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Effects of lipids on inflammation and DNA damage in diabetes mellitus patients

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Abstract

We aimed to see the effect of lipids on 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of DNA damage, and Interleukin-6 (IL-6), an inflammatory cytokine, in Diabetes Mellitus (DM) patients. Serum IL-6, and 8-OHdG levels were determined using kits based on the ELISA principle. The groups were different in terms of total cholesterol, LDL cholesterol and triglyceride. Group 2 (dyslipidemia positive DM positive) was significantly higher than the other groups. There was no statistical difference between group 1 (dyslipidemia negative DM positive) and the control group ($p<0.001$). 8-OHdG levels differ between groups. The level of 8-OHdG was significantly higher in group 2 compared to the other groups ($p<0.001$). IL-6 was different between groups. Group 2, IL-6 level was found to be significantly higher than the other groups ($p<0.001$). A positive correlation was found between serum 8-OHdG level and total cholesterol ($r=0.417$ $p<0.001$), LDL cholesterol ($r=0.491$ $p<0.001$) and triglyceride ($r=0.441$ $p<0.001$). A positive correlation was found between IL-6 and total cholesterol ($r=0.522$ $p<0.001$), LDL cholesterol ($r=0.646$ $p<0.001$) and triglyceride ($r=0.495$ $p<0.001$). IL-6 and 8-OHdG levels are high in dyslipidemic DM patients with a high lipid profile. IL-6 and 8-OHdG levels are elevated in dyslipidemic DM patients with a high lipid profile. IL-6 and 8-OHdG can be used as biomarkers to evaluate dyslipidemic complications seen in DM patients in the early diagnosis.

Keywords: Diabetes mellitus, DNA damage, dyslipidemia, interleukin 6, 8-hydroxy-2'-deoxyguanosine

Introduction

Diabetes mellitus (DM) is a global health problem and its prevalence is increasing every year [1]. According to the data of the International Diabetes Federation, approximately 537 million people in the world have diabetes. It is estimated that this number will increase to 643 million in 2030 [2].

DM is not only a carbohydrate metabolism disorder, but also a lipid and protein metabolism disorder. A dyslipidemia characterized by high triglyceride levels, low high-density lipoprotein (HDL) cholesterol and high low-density lipoprotein (LDL) cholesterol ratios is seen in DM. The increased risk of cardiovascular disease in individuals with DM is partially clarified by the lipoprotein abnormalities seen this disease [1,3].

It has been shown that increases in blood sugar levels in DM disease increase free radicals and cause oxidative stress [4,5]. The exist of oxidative stress is known to play an major role in DM and DM-related diseases such as dyslipidemia, hypertension and atherosclerosis [6,7]. Reactive oxygen species (ROS) can cause deletions of different sizes, insertions, DNA base damage and strand breaks [8,9]. Compared with other bases, guanine is most susceptible to oxidation by ROS [10]. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is among the most extensively studied biomarkers of oxidative DNA damage in humans, formed by guanine oxidation [9,11,12].

ROS is also very active in the progression of inflammatory disorders [13]. Interleukin-6 (IL-6) is a proinflammatory cytokine secreted from monocytes, macrophages, fibroblasts,

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and endothelial cells that is activated in response to stimuli such as oxidative stress, bacterial endotoxins, and physical exercise [14,15]. It was reported that inflammatory cytokines such as IL-6 are related with diabetes development [16].

Although there are studies investigating the effect of lipids in DM patients in the literature, the number of studies investigating the effects on inflammation and oxidative DNA damage is limited. Therefore, in this study, we investigated to see whether lipids caused DNA damage and inflammation in people with diabetes.

Material and Methods

Study design

In this study, blood samples were taken from the patients who applied to the Education and Research Hospital, Department of Internal Medicine, which is an affiliated institution to Ordu University Faculty of Medicine, by signing a consent form.

A total of 84 participants, case and healthy control, were included in this study. The case group consisted of people over the age of 18 with a diagnosis of DM. DM patient group consists of two groups as dyslipidemia negative (referred to as Group 1, comprising 22 cases) and dyslipidemia positive (referred to as Group 2, comprising 32 cases). The control group (30 person) consists of people who are not diagnosed with DM. Cases with malignancy, autoimmune disease, taking vitamin therapy, using glitazone and statin were not included in the study.

Blood samples were taken from the individuals included in the study. The serum was separated by centrifugation for 20 minutes. The serum samples were stored at -80ºC until use.

Ethical approval for the study was obtained from the Ordu University Faculty of Medicine Institutional Review Board Ethics Committee (dated: 18.02.2021, reference number: 2021/KAEK 42). All subjects provided informed consent prior to their inclusion in the study.

Laboratory analysis

The serum levels of IL-6 and 8-OHdG were assessed utilizing enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocols. For IL-6 measurement, the DIA Source ImmunoAssays S.A kit from Belgium was employed, while the Cloud-Clone Corp kit from the USA was used for 8-OHdG analysis. The protocols of the relevant kits were Biotek Epoch 2 microplate reader was used for absorbance values (450 nm). Results were analyzed using Gen5 software.

The values of 8-OHdG and IL-6 in the serum were computed from the standard curve. The outcomes were expressed as pg/ml. The detection range for 8-OHdG was 74.07 to 6.000 pg/ml, while for IL-6, it was 0 to 2560 pg/ml.

Statistical analysis

The data were tested in terms of normality with Kolmogorov-Smirnov test. Normally distributed data were analyzed with One-Way Anova test and non-normally distributed data were compared with Kruskal Wallis test. Categorical data were compared using the Chi-Square test. In the chi-square tests, if a cell had an expected frequency below 5, likelihood ratio chi-square value was applied instead of Pearson chi-square value. The numeric variables as mean±SD and median (min-max), the categorical variables as percentage were expressed. Pearson correlation test was used for normally distributed data and Spearman correlation test was utilized for data not normally distributed. SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software was used. The results were appraised based on the 95% confidence interval and the level of significance was $p < 0.05$.

Results

When the groups were compared in terms of gender, smoking, and chronic diseases, the rate of hypertension was significantly lower in the control group ($p<0.001$). Hypertension (HT) rates in group 1 (HT 65.6%) and group 2 (HT 63.6%) were similar. There was no significant statistical difference between the groups in terms of gender, other chronic diseases and smoking (Table 1).

Table 1. Comparison of gender and chronic diseases of the groups

	Group 1 DM (+) Dyslipidemia (-)	Group 2 DM (+) Dyslipidemia (+)	Control group DM (-) Dyslipidemia (-)	P value
Gender (%)				
Male	65.6	63.6	80.0	0.341
Female	34.4	36.6	20.0	
Hypertension (%)	65.6	63.6	16.7	**<0.001**
Thyroid diseases (%)	25.0	18.2	23.3	0.831
Coronary artery disease (%)	3.1	0	0	0.377
Heart failure (%)	3.1	4.5	0	0.393
Chronic kidney disease (%)	3.1	0	0	0.377
Cigarette (%)	18.8	22.7	23.3	0.894

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When the groups were compared in terms of age and laboratory tests; found to differ between age groups (Table 2). The mean age of the 1st group was 56.19±11.58, and the mean age of the 2nd group was 53.09±8.55, the mean age of the control group was 40.33±12.9. While there was no difference in age between group 1 and group 2, the mean age of the control group was lower (p<0.001). Fasting glucose was different in all 3 groups. Fasting glucose was highest in group 2 and lowest in control group (p<0.001). There were also differences between the groups in terms of alanine aminotransferase (ALT) and aspartat aminotransferase (AST) levels. While there was no difference between group 1 and group 2, it was significantly lower in the control group (p<0.001 p=0.014, respectively). The groups were different with respect to total cholesterol, LDL cholesterol and triglyceride. Group 2 was different from other groups. There was no difference between group 1 and control group (p<0.001) (Table 2).

8-OHdG levels differ between groups. The level of 8-OHdG was significantly higher in Group 2 compared to the other groups (p<0.001). IL-6 was different between groups. Group 2 IL-6 level was found to be significantly higher than the other groups (p<0.001) (Table 2).

A positive correlation was determined between serum 8-OHdG level and total cholesterol (r=0.417 p<0.001), LDL cholesterol (r=0.491 p<0.001) and triglyceride (r=0.441 p<0.001) (Table 3). A positive correlation was found between IL-6 and total cholesterol (r=0.522 p<0.001), LDL cholesterol (r=0.646 p<0.001) and triglyceride (r=0.495 p<0.001) (Table 4).

Table 2. Comparison of age and laboratory tests of groups

	Group 1 DM (+) Dyslipidemia (-)	Group 2 DM (+) Dyslipidemia (+)	Control group DM (-) Dyslipidemia (-)	P value
Age	56.19±11.58	53.09±8.55	40.33±12.9	**<0.001[a]**
Hemoglobin. g/dl	13.7±1.51	13.9±1.49	13.6±1.44	0.824
White blood cell. 10^3 uL	7.47±2.27	7.28±1.95	7.01±1.44	0.654
Platelet. 10^3 Ul	264.56±92.34	270.64±68.29	257.97±67.10	0.377
Fasting blood glucose. mg/dl	141.0 (94-211)	165.0 (111-331)	97.0 (81-117)	**<0.001[c]**
Creatinine. mg/dl	0.82±0.29	0.72±0.16	0.69±0.13	0.051
Blood urea nitrogen. mg/dl	14.8±6.32	13.20±5.26	11.51±4.49	0.058
Aspartat aminotransferase. IU/l	21.0 (12-61)	21.0 (8-74)	15.0 (8-24)	**0.014[a]**
Alanine aminotransferase. IU/l	24.0 (5-68)	24.0 (11-77)	13.0 (8-22)	**<0.01[a]**
Total bilirubin. mg/dl	0.52±0.22	0.49±0.31	0.51±0.30	0.936
CRP. mg/dl	0.61±1.01	0.84±1.40	0.28±0.35	0.121
Total cholesterol. mg/dl	182.5±31.4	257.5±4.98	187.35±41.01	**<0.001[b]**
LDL-cholesterol. mg/dl	101.5±31.2	163.6±65.5	105.8±38.3	**<0.001[b]**
HDL-cholesterol. mg/dl	51.37±12.3	44.83±13.0	50.28±12.2	0.150
Triglyceride. mg/dl	155.6±59.7	330±249.37	123.8±74.60	**<0.001[b]**
TSH. mU/L	2.32±1.37	2.23±1.51	2.25±1.22	0.965
8-OHdG	74.07 (74.07-81.27)	205.0 (74.07-919.35)	74.07 (74.07-74.07)	**<0.001[b]**
IL-6	4.09 (3.30-5.56)	8.23 (4.17-56.31)	3.35 (2.43-3.84)	**<0.001[b]**

a: There is a difference between the control group and the other groups, b: There is a difference between group 2 and other groups, c: Different from each other in all 3 groups

Table 3. Correlation between 8-OHdG and lipid profile

	r	p
Total cholesterol, mg/dl	**0.417**	**<0.001**
LDL-cholesterol, mg/dl	**0.491**	**<0.001**
Triglyceride, mg/dl	**0.441**	**<0.001**

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Table 4. Correlation between IL-6 and lipid profile

	r	p
Total cholesterol, mg/dl	**0.522**	**<0.001**
LDL-cholesterol, mg/dl	**0.646**	**<0.001**
Triglyceride, mg/dl	**0.495**	**<0.001**

Discussion

Genome stability is crucial for normal cell physiology. Increased oxidative stress makes DNA bases highly susceptible to damage [17]. DM and its associated diseases are related with increased levels of oxidative stress. According to the previous studies, it has been determined that lipids have an major role in the activation of inflammatory pathways and increase the production of inflammatory cytokines such as IL-6 [18]. In this study, we aimed to examine the effects of lipids on IL-6 and 8-OHdG, which are markers of inflammation and oxidative DNA damage, in DM patients. To our knowledge, no study investigating the effect of lipids on serum IL-6 and 8-OHdG levels in DM patients has yet been reported. Our research represents the novel investigation into this particular area.

In this study, we found fasting glucose, total cholesterol, LDL cholesterol and triglyceride levels to be higher in the dyslipidemic diabetes group. In the study of Yong Hoon Kim et al.; HDL and LDL cholesterol levels were lower in the dyslipidemic diabetes group than in the control group. However, triglyceride was higher in the dyslipidemic diabetes group. In the same study, the total cholesterol level was higher in the control group, and the fasting glucose level was higher in the diabetes-positive dyslipidemia-negative group [19]. The prominent features of diabetic dyslipidemia include high triglyceride, low HDL, and increased LDL cholesterol concentrations. Lipid changes associated with DM are connected to increased free fatty acid efflux secondary to insulin resistance [20,21]

Jameil et al. reported that determinants of liver function tests increased with hyperlipidemia in type 2 diabetes patients [22]. In this study, we could not find a meaningful difference between diabetes and dyslipidemic diabetes groups in AST and ALT levels, and we determined it to be lower in the control group. There are very few studies in the literature with dyslipidemic DM patients. Our study is compatible with the literature within the data we can reach.

In this study, we found that IL-6 and 8-OHdG levels in the dyslipidemic diabetes patient group were significantly different from the diabetes and control groups. A study conducted by Krysiak et al., in male and female with type 2 diabetes with atherogenic dyslipidemia, there was no statistical difference in serum IL-6 levels [23]. Paul-Mihai Boarescu et al. studied cardiovascular risk factors in their study with rats and determined that IL-6 level was higher in the dyslipidemic patient group compared to the control group [18]. However, in the work of Mohammed H. Mukhtar et al.; They found IL-6 and 8-OHdG levels to be higher in DM cases compared to the control group. However, only the DM case group was studied in this study [24]. There are a limited number of studies investigating the effects of dyslipidemia in the literature, and often only DM patients have been studied. In these studies, IL-6 and 8-OHdG levels are generally high in type 1 and type 2 diabetes cases [17,24].

In this study, a positive relationship was determined between IL-6 and 8-OHdG and lipid parameters triglyceride, total cholesterol and LDL cholesterol. Due to the increasing morbidity and mortality rate, DM requires additional preventive and therapeutic strategies. There is a need for new biomarkers that can be used in treatment control and risk assessment as an alternative to existing methods. DM is an inflammatory process that accompanies comorbidities [25]. Lipids are highly active in the activation of inflammatory pathways. Dyslipidemia is an important determinant of the development of atherosclerosis, which is very important for endothelial damage [19,26].

Conclusions

IL-6 and 8-OHdG levels are high in dyslipidemic DM cases with a high lipid profile. Based on this, 8-OHdG and IL-6 can be used as biomarkers to evaluate dyslipidemic complications seen in DM patients in the early diagnosis. However, longer follow-up and studies in larger case populations are required to better see the clinical effects of lipids in DM.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
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Ethical Approval
This study was approved by the Ordu University, Faculty of Medicine Institutional Review Board Ethics Committee (date: 18.02.2021 number: 2021/KAEK 42).

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