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Evaluation of interleukin-33 and osteopontin levels in well and poorly regulated diabetes mellitus type 2

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Abstract

IL-33 is one of the novel IL-1 family members which are secreted in many tissues. Osteopontin (OPN) is a highly phosphorylated and glycosylated sialoprotein synthesized primarily in bone tissue, which the major role is to participate in biomineralization. Both IL-33 and OPN are mentioned associated with inflammatory disorders. Diabetes Mellitus Type 2 (T2DM) is a metabolic disorder in glucose homeostasis, which its pathophysiology involves a low-grade chronic inflammation. The aim of this study is to evaluate the IL-33 and OPN levels in well and poorly regulated T2DM patients. Three group were determined as; T2DM with HbA1c level below 8% as Group HbA1c <8, T2DM with HbA1c level of 8% and above as Group HbA1c ≥8, and control group consisting of healthy individuals respectively. Each group included 50 participant (n=50). Age, fasting plasma glucose, HbA1c and lipid parameters (total cholesterol, triglyceride, LDL, HDL), IL-33 and OPN values were examined. There was a significant statistical difference between the fasting plasma glucose, HbA1c, triglyceride, and HDL levels of the groups (p<0.001). OPN levels of the groups were not found statistically different but IL-33 levels were found to be significantly higher in the HbA1c <8 group compared to the control group (p=0.0108). IL-33 and OPN levels do not differ in terms of well or poorly regulation in T2DM patients and HbA1c level alone, is insufficient to be a predictor of OPN and IL-33 activity.

Keywords: IL-33, osteopontin, hbA1c, diabetes mellitus type 2

Introduction

Natural inflammatory responses are mediated by cytokines. IL-1 family cytokines and their receptors show pro-inflammatory and anti-inflammatory activities in human body. IL-33 is one of this family cytokines, and it was first identified in 2005 [1]. It is known to be produced in mucosal epithelium, fibroblasts, keratinocytes, and endothelial cells [2]. In addition, it has been discovered that cells of hematopoietic origin as mast cells, dendritic cells, macrophages and monocytes, also secrete IL-33 [3]. Suppressor Tumorogenicity (ST2) is an interleukin-33 receptor which is secreted in reaction to cell damage. There are two primary versions of ST2: the membrane-bound/cellular form (ST2L) and the soluble/circulating form (sST2). The circulating sST2 version can be significantly triggered in living cells and is produced by nearly all tissues. sST2 competes with ST2L for binding to IL-33, leading to inhibition of the IL-33/ST2L system. Thus, increased levels of IL-33 serve as a marker for inflammation and tissue necrosis, while sST2 acts as a response to inflammation. In recent years, research has connected the IL-33-ST2 axis with human health and metabolic diseases [4]. Nuclear expression of IL-33 is notably high in lung epithelial barrier tissue and plays a role in adaptive immune responses by mediating induced airway hyper-reactivity when exposed to allergens or infections [5]. Abnormal expression of IL-33 was observed in certain conditions such as rheumatoid arthritis, inflammatory bowel disease, Behçet's disease, and atopic dermatitis, heightening its importance. [6]. By the stimulation of IL-33, sST2 is secreted from the endothelial cells of the aorta, coronary arteries, cardiac fibroblasts and cardiomyocytes. Because of the elevation of serum sST2 levels

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after the transmural myocardial injury, sST2 was associated with the prognosis of coronary artery disease. Again, higher plasma sST2 concentrations were found in hypertensive patients with left ventricular hypertrophy, furthermore sST2 was stated as a biomarker for the prognosis of heart failure [7].

Osteopontin (OPN) is a glycosylated sialoprotein synthesized principally in bone tissue. Its primary physiological role is to participate in bone mineralization and calcification processes. It is also released in many tissues such as the kidney, brain, smooth muscle, skeletal muscle, biliary and pancreatic tracts [8]. OPN has also a role in the regulation of the extracellular matrix in cellular immunity as cellular infiltration and tissue repair, also in inflammatory diseases and angiogenesis [9]. Some studies showed the association of OPN with the apoptosis inhibition and tumor metastasis so with the progression of different malignancies [10]. Again, some studies stated that OPN expression is elevated in acute and chronic kidney diseases [11]. Furthermore, it was found associated with the pathogenesis of acute coronary syndrome, cardiomyocyte hypertrophy and fibroblast proliferation because its higher expression was observed in foam cells which are accepted as the marks of atherosclerotic plaques. That's why OPN is generally defined as a proinflammatory and proatherogenic molecule [12].

Type 2 diabetes mellitus (T2DM) is characterized by disruptions in glucose balance due to inadequate or abnormal insulin secretion, insulin resistance, and high levels of blood glucose. It also involves low-grade chronic inflammation that contributes to microvascular and macrovascular complications, making T2DM a cardiovascular risk factor [13]. In this study, we aimed to investigate OPN and IL-33 levels in relation to fasting blood glucose, lipid parameters, HbA1c levels in both well-controlled and poorly controlled T2DM patients as well as healthy control groups. There has been yet no study evaluating OPN and IL-33 together in the context of T2DM. According to the results, changes in OPN and IL-33 levels will be evaluated in terms of inflammation and immune reactions and their properties as regulation markers in T2DM will be questioned.

Material and Methods

Study Design

The study was conducted within the scope of patients who applied to the internal medicine outpatient clinic of Training and Research Hospital of Medicine Faculty of University Ordu. Individuals between the ages of 18 and 75 were included in the study. The patient group consisted of patients who were followed up due to T2DM. All the diabetic patients were using oral antidiabetic drugs. The control group consisted of patients who applied with any complaints. Those with active infections, pregnancy status, suspicion of malignancy, systemic disease other than T2DM, and medication use for any reason were excluded from the study. Three groups were defined in the study; Group HbA1c<8; T2DM patients with HbA1c level below 8%, Group HbA1c≥8; T2DM patients with HbA1c level of 8% and above, and control group as healthy individuals. Fifty participants for each group were included in the study and all participants gave their written informed consent.

The study was approved by The Clinical Research Ethics Committee of Ordu University (11.02.2022/2022/34) and has been carried out following principles endorsed by the Declaration of Helsinki.

Biochemical Analyzes

Fasting plasma glucose, HbA1c, lipid parameters (including total cholesterol, triglycerides, VLDL, LDL and HDL), IL-33 and OPN levels were assessed. These measures were conducted during routine follow-up tests for T2DM patients and in response to complaints for the control group. Serum samples were obtained from venous blood collected after an overnight fast, followed by centrifugation at 1800 x g for 10 minutes to isolate the serum components. The separated serum was stored at -80°C until analysis. Measurement of IL-33 and OPN levels was carried out using ELISA method (Nepenthe IL-33 elisa kit Kocaeli/Türkiye, Lot no: NE06S330903. Detection limit: 4.7 pg/mL and Nepenthe Osteopontin elisa kit Kocaeli/Türkiye, Lot no: NE06C521802, Detection limit: 0.026 ng/mL respectively) at the Biochemistry Laboratory of Medicine Faculty of University Ordu.

Statistical Analysis

Statistical Package for Social Sciences (SPSS, version 25.0, IBM Inc. Armonk, USA) was used for the data analysis. Descriptive statistics are expressed as mean, standard deviation, minimum and maximum for continuous variables. Categorical variables are expressed as number and percentage. Kolmogorov-Smirnov test was used to determine whether the groups were suitable for normal distribution. The Kruskal-Wallis test and One-way ANOVA were used to compare the three groups. In pairwise comparison of groups, Student-t test was used for groups with normal distribution, and Mann-Whitney U test for groups without normal distribution. Spearman rho test was performed to evaluate the correlation between HbA1c and IL-33 and OPN. Groups are shown graphically as mean±2SD or with Box-whisker plots. The value $p<0.05$ was considered statistically significant.

Results

The mean age of the control group was 41.14±15.39, and it was significantly younger than both T2DM groups ($p<0.001$). There was a significant difference between the groups in terms of fasting blood glucose, HbA1c, triglyceride, VLDL and HDL levels ($p<0.001$). Descriptive parameters and laboratory results of the groups were given in Table 1. The triglyceride and HDL levels according to groups were shown in Figure 1. When compared with Independent t-Test; OPN level was not statistically different between groups but IL-33 was found to be significantly higher in Group HbA1c<8, compared to the control group ($P=0.0108$). (Figure 2). Spearman rho test was performed to evaluate the correlation between HbA1c and IL-33 and OPN. Spearman correlation coefficient was -0.019, $p=0.818$ and -0.023, $p=0.777$. between HbA1c and IL-33 and between HbA1c and OPN respectively.

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Table 1. Comparison of age and laboratory parameters of the groups

	Control group (n=50) Mean±SD	Group HbA1c <8 (n=50) Mean±SD	Group HbA1c ≥8 (n=50) Mean±SD	p value
Age	41.14±15.39 (20-79)	57.62±11.01 (22-77)	54.54±13.45 (29-78)	<0.001
Fasting Blood Glucose (mg/dL)	93.34±6.94 (77-109)	122.48±29.91 (90-245)	251.68±97.58 (103-547)	<0.001
HBA1c (%)	5.51±0.34 (4.8-5.5)	6.55±0.65 (5.5-7.9)	10.19±1.64 (8-15.4)	<0.001
Cholesterol (mg/dL)	192.46±63.7 (108-528)	203.08±46.95 (112-354)	214.66±51.86 (117-321)	0.131
Triglyceride (mg/dL)	111.76±56.35 (41-301)	158.34±77.83 (38-470)	243.44±190.13 (72-1191)	<0.001
HDL-C (mg/dL)	53.66±13.09 (29.7-101.6)	50.46±11.94 (31-81.3)	44.04±9.01 (24.3-66.2)	<0.001
LDL-C (mg/dL)	110.84±35.44 (42.3-205.3)	122.03±38.09 (44.1-249.2)	121.88±39.29 (44-210.7)	0.237
VLDL-C (mg/dL)	22.37±11.42 (8.2-60.2)	30.92±15.34 (7.6-90)	48.38±41.72 (14.4-238.2)	<0.001
IL-33	55.95±79.47 (3.04-507.52)	85.04±116.60 (2.78-515.47)	59.08±85.56 (7.92-500.85)	p>0.05* p=0.0108**
OSPN	1.43±2.69 (0.02-17.78)	1.76±3.31 (0.03-19.69)	0.93±1.26 (0.01-6.20)	p>0.05

*: Comparison of groups HbA1c <8 and HbA1c ≥8, and control and HbA1c ≥8, **:Comparison of HbA1c <8 and control groups, P<0.05 value was considered statistically significant

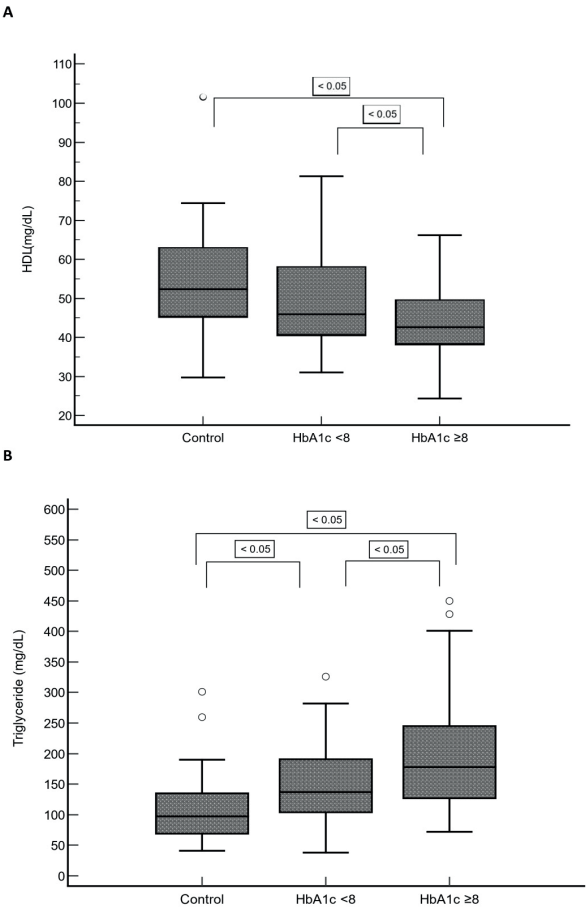

Figure 1. The comparison of the HDL (A) and triglyceride (B) levels of the groups

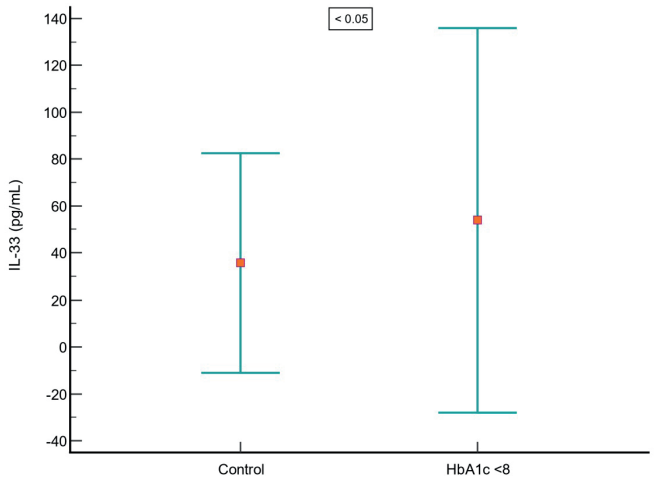

Figure 2. The comparison of IL-33 levels in control and HbA1c<8 groups

Discussion

Cytokines are important mediators of the immune system. In addition, they are also associated with metabolic and inflammatory disorders [14]. IL-33 acts on cells where the T1/ST2 receptor is present, causing the release of pro-inflammatory leukotrienes IL-6 and TNF-alpha. This status results as the increased microvascular permeability and the accumulation of inflammatory cells [15]. In recent years, studies have been conducted on the relationship between IL-33 and fat tissue. These studies reveal different results. In one study, it was stated that IL-33 is especially effective in obesity and insulin resistance via its relation with adipose tissue inflammation [16].

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Again, it was determined that IL-33 increased in fat tissue with insulin resistance and diabetes and inhibited glucose uptake into fat tissue cells. IL-33 mRNA expression was found to have a positive correlation with HbA1c and body mass index [17]. In contrast, a study in mice revealed that IL-33 stimulated the expression of its receptor ST2 and the transcription factor PPARγ. Additionally, under hyperglycemic conditions, IL-33 contributed to the protection of cell viability and insulin secretion in pancreatic β-cells [18]. Moreover, a research demonstrated increased adipose tissue in mice lacking IL-33, while treatment with IL-33 resulted in reduced adipose tissue inflammation and insulin resistance. The administering rIL-33 to genetically obese diabetic mice led to decreased adiposity and fasting plasma glucose levels as well as improved insulin tolerance [19]. It is stated that IL-33 functions as an anti-inflammatory cytokine in many inflammatory events with increasing levels [20]. One study found that diabetic individuals had much lower IL-33 concentrations than healthy individuals [21]. In another study, IL-33 expression in the adipose tissue of T2DM patients was found to be inversely associated with glycemia, but no difference was observed depending on whether HbA1c levels were higher or lower. This result has been interpreted as indicating that the IL-33/ST2 axis has a protective function in the early stage of T2DM and that this could potentially change with the use of glucose-lowering therapies [22]. Similarly, in our study, IL-33 levels were not found to be different between well and poorly regulated T2DM patients. It was determined that IL-33 levels increased in patients with HbA1c levels below 8% compared to non-diabetic individuals. This result may support the view that IL-33 increases versus the inflammation in T2DM and, it is an ameliorating response to glucose dysregulation in mild T2DM.

Although OPN is found in many tissue cells, it is especially found in bone tissue and its main function is to manage bone mineralization [23]. Recent studies showing its relationship with cardiovascular diseases contain important results. One study found that OPN demonstrated aortic valve calcification in asymptomatic individuals [24]. Another study found that OPN showed early coronary calcification in asymptomatic T2DM patients [25]. Similarly, another study stated that OPN may be a marker of inflammation-related heart diseases [26]. Looking at the relationship between OPN and adipose tissue, OPN was found to stimulate inflammatory signaling pathways and induce cytokine release in human adipose tissue cells and macrophages [27]. It has also been mentioned that OPN plays a role in the pathophysiology of inflammation, obesity and insulin resistance [28]. It has been shown that the differentiation of fat cells and insulin sensitivity are impaired by OPN, and therefore OPN causes insulin resistance [29]. Some studies have similarly shown that OPN levels in blood and adipose tissue increase in obesity, obese diabetic patients and insulin resistance [30,31]. It is noteworthy that studies with high OPN levels are especially studies involving obesity and insulin resistance. There is no study in the literature showing that OPN increases in diabetic patients, regardless of obesity or insulin resistance. In our study, our T2DM patients were not divided according to obesity degree and, OPN levels of diabetic and non-diabetic individuals were not found to be different.

Both IL-33 and OPN have been evaluated in some studies for their association with diabetic complications. In a study, it was mentioned that the IL-33/ST2 axis was effective in nephropathy in diabetic patients [32]. Another study in diabetic mice showed that IL-33 is a potent cardioprotector and treatment with IL-33 improved cardiac diastolic function [33]. OPN levels were discovered to be notably elevated in T2DM patients with microvascular complications when compared to those without such complications [34]. Another study observed higher OPN levels in patients with diabetic retinopathy than in those without this complication [35]. However, in contrast to these results, in one study OPN levels increased significantly with progression of diabetic nephropathy but did not change with the progression of diabetic retinopathy or diabetic neuropathy [36]. HbA1c level indicates the degree of regulation in T2DM and is considered a predictive parameter for the development of complications in T2DM. However, high and continuous HbA1c levels rather than short-term increases are effective in the development of complications [37]. Therefore, complications do not always occur in parallel with the HbA1c level. Age, duration of T2DM, and systemic diseases also contribute to the formation of complications. In this study, it is not known how long HbA1c levels were high in the patients. The significant difference between the ages of the diabetic and control groups, the gender factor, different medications used are the limitations of this study.

Conclusion

The roles of IL-33 and OPN are not clear in the pathophysiology of T2DM. IL-33 and OPN levels do not differ in terms of well or poorly regulation in T2DM patients and HbA1c value alone, is insufficient to be a predictor of OPN and IL-33 activity.

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 Clınıcal Research Ethıcs Commıttee of Ordu University (11.02.2022/ 2022/34) and has been carried out following principles endorsed by the Declaration of Helsinki.

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