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Assessment of endothelial dysfunction and inflammation in type 2 diabetic postmenopausal women

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ABSTRACT

Objective: Vascular complications in type 2 diabetes mellitus are an important cause of morbidity and mortality. Also, endothelial dysfunction arises with vascular ageing during the postmenopausal period. Our objective in this study was to evaluate inflammation and endothelial function parameters and their possible diagnostic roles in type 2 diabetic postmenopausal patients.

Material and Methods: The study was conducted on four groups, including type 2 diabetic premenopausal (n:20), non-diabetic premenopausal (n:20), type 2 diabetic postmenopausal (n:20), and non-diabetic postmenopausal subjects (n:20). Serum endothelin-1 (ET-1), endothelial nitric oxide synthetase (eNOS), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and chitinase-3-like protein 1 (YKL-40) levels were determined as inflammatory and endothelial function markers using ELISA kits.

Results: Serum ET-1, IL-6, and YKL-40 levels were higher in the type 2 diabetic postmenopausal group compared to the non-diabetic premenopausal group ($p<0.01$, $p<0.05$, and $p<0.01$, respectively). ET-1, IL-6, and YKL-40 levels were higher in the type 2 diabetic postmenopausal group compared to the non-diabetic postmenopausal group ($p<0.001$, $p<0.05$, and $p<0.01$ respectively). ROC analysis revealed serum ET-1 (AUC 0.933, sensitivity 87.5%, specificity 85.7%), IL-6 (AUC 0.812, sensitivity 56.3%, specificity 92.8%), and YKL-40 (AUC 0.880, sensitivity 81.2%, specificity 92.8%), as good diagnostic parameters, especially in the type 2 diabetic premenopausal vs. non-diabetic premenopausal cohorts.

Conclusion: Serum ET-1, IL-6, and YKL-40 levels were at the highest levels in the 2 diabetic postmenopausal group, and the increase in these markers was remarkable in diabetes compared with menopausal periods. Also, ET-1, IL-6, and YKL-40 were good diagnostic parameters for detecting endothelial function and inflammation in type 2 diabetic postmenopausal and non-diabetic premenopausal cohorts.

Keywords: Type 2 diabetes mellitus; menopause; endothelial function; inflammation.

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INTRODUCTION

Menopause is a normal physiologic event reflecting ovarian follicular function loss, oestrogen and progesterone levels decline, amenorrhoea continues for a year, and the reproduction period ends (1). Besides increasing somatic and psychological symptoms in the menopausal period, vascular ageing becomes faster. Arterial stiffening and endothelial dysfunction form with vascular ageing (2).

Type 2 diabetes mellitus is an important progressive disease with a prevalence increasing with age. Endothelial-dysfunction-related micro-and macrovascular complications occur in type 2 diabetes mellitus, which is an endocrinal metabolism disease characterised by chronic inflammation (3). Endothelial dysfunction is characterised by three main metabolic disorders in type 2 diabetes. These are hyperlipidaemia, early hyperinsulinemia, and pancreatic cell deficiency causing hyperglycaemia after hyperinsulinemia (4).

Endothelial tissue dysfunction is related to the imbalance of vasoconstrictor and vasodilator molecules secreted by the endothelium. Endothelin-1 (ET-1) is the strongest vasoconstrictor molecule functioning in the provision of molecule exchange order of endothelial tissue (5). Endothelium-derived synthesised nitric oxide (NO) takes functions in vascular tone regulation, leukocyte adhesion, aggregation of thrombocytes, and proliferation of vascular smooth muscles. NO synthesis from L-arginine and oxygen in endothelial cells is conducted through the endothelial nitric oxide synthase (eNOS) enzyme (6).

The development of atherosclerosis among vascular pathologies depends on the changing behaviour of endothelial cells and vascular smooth muscle cells. Inflammation is an important risk factor in the development of atherosclerosis (7). Tumor necrosis factor α (TNF-α) is known to be a potent promoter of inflammation, as well as many normal physiological functions in homeostasis. It is secreted in the acute phase of many inflammatory reactions and is produced in macrophage, endothelial and adipose tissues. It has been reported that TNF-α is an important proinflammatory cytokine, is associated with recurrent coronary artery occlusions. (8).

Interleukin-6 (IL-6) is mostly regarded as a pro-inflammatory cytokine, but it also has many regenerative or anti-inflammatory activities. As a multifunctional cytokine, IL-6 was shown to increase the secretion of endothelial-derived adhesion structures. It is an important independent molecule that is considered effective in risk determination in cardiovascular events (3, 9).

YKL-40 protein, known as chitinase-3-like protein 1 (CHI3L1) is secreted by vascular smooth muscle and macrophage cells, and stimulated for natural immune response realisation. It has been reported that the It has been reported that the YKL-40 may play a role in endothelial dysfunction and atherosclerosis (3).

Considering this information, we reviewed the literature and saw that there were studies reporting inflammation and endothelial function in type 2 diabetes mellitus, and in the menopause period, but studies evaluating the menopausal period and type 2 diabetes mellitus were limited. Also, the mechanism of vascular pathogenesis forming in this period is not completely clear yet. Thus, our objective in this study was to investigate serum ET-1, eNOS, IL-6, TNF-α, and YKL-40 levels in pre and post-menopausal women with and without type 2 diabetes. Also, we evaluated the diagnostic performance of these markers for detecting inflammation and endothelial function in non-diabetic, and subjects with diabetes in pre- and postmenopausal status.

MATERIAL and METHODs

Case selection: This study was conducted on a total of 80 individuals including postmenopausal type 2 diabetic (n:20), postmenopausal non-diabetic (n:20), premenopausal type 2 diabetic (n:20), premenopausal non-diabetic (n:20) individuals, who applied to Suluntepe Family Health Center. The present study was approved by the Istanbul University-Cerrahpasa Medical Faculty Ethics Committee (approval number: 332891/2017) and was performed in accordance with The Declaration of Helsinki. All participants gave written informed consent.

The individuals with amenorrhoea constituted postmenopausal groups. Type 2 diabetic patients were selected according to the Endocrinology and Metabolism Society's Diagnosis, Treatment, and Follow-up Guidelines for Diabetes Mellitus and Complications. During the last six months, the participants have not used any hormonal contraceptives, also during the last four weeks, they have not used antihypertensive, lipid-lowering, and anti-inflammatory drugs. Moreover, they did not undergo hysterectomy or oophorectomy operation and did not have any chronic diseases.

Sample collection: Five milliliters of venous blood samples were taken after 12 h of fasting from all individuals. Blood samples were centrifuged at 3000 x g for 10 min, and the obtained serum samples were kept at −80°C until analysis. ET-1, eNOS, IL-6, TNF-α, and YKL-40 levels were determined in serum samples. Glucose, HbA1c, insulin, and insulin resistance levels were routine markers examined in Istanbul Public Health Laboratory. Homeostatic Model Assessment Score (HOMA) was used to determine insulin resistance (1).

Measurement of endothelial function and proinflammatory markers: The levels of ET-1, eNOS, IL-6, TNF-α, and YKL-40 in serum samples were quantified according to the manufacturer's instructions and guidelines using ELISA kits (Boster Bio, Pleasanton, CA, USA, for all).

Statistical evaluation: Data were presented as mean ± the standard deviation (SD). After checking for normality assumptions with the Shapiro-Wilk normality test, statistical significance was determined using one-way ANOVA followed by Holm-Sidak's multiple comparison test or Kruskal-Wallis test with Dunn's multiple comparisons. Receiver operating characteristic (ROC) analysis and area under the ROC curve (AUC) was used the determination of the diagnostic performance of inflammation and endothelial function markers. Statistical Package for the Social Sciences-SPSS 21.0 for Windows (SPSS Inc, Chicago, IL, USA) was used for calculations. $p<0.05$ was considered to indicate a statistically significant difference.

RESULTs

Demographic Data

Age, body mass index (BMI), duration of menopause, duration of diabetes, systolic blood pressure (SBP), diastolic blood pressure (DBP), glucose, HbA1c, insulin, and insulin resistance values of all groups were presented in Table 1 as the mean ± SD. No significant differences were found in age and BMI values between the type 2 diabetic premenopausal and the non-diabetic premenopausal groups. Age values were higher in the non-diabetic postmenopausal than the non-diabetic premenopausal group ($p<0.001$). Also, the age results were increased in the non-diabetic postmenopausal group compared with the type 2 diabetic premenopausal group ($p<0.01$). The values of the type 2 diabetic postmenopausal group were increased than the non-diabetic, and type 2 diabetic premenopausal groups ($p<0.001$, for both).

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SBP, DBP, glucose, HbA1c, insulin, and insulin resistance results were higher in the type 2 diabetic premenopausal group than the non-diabetic premenopausal group ($p<0.01$, $p<0.05$, $p<0.001$, $p<0.001$, $p<0.01$, and $p<0.001$, respectively). SBP, glucose, HbA1c, insulin, and insulin resistance results were higher in the type 2 diabetic postmenopausal group than the non-diabetic postmenopausal group ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.01$, and $p<0.001$, respectively). Insulin, and insulin resistance levels increased in the non-diabetic postmenopausal group compared with the non-diabetic premenopausal group ($p<0.05$, for both). The glucose, HbA1c, and insulin resistance values of the type 2 diabetic postmenopausal group were increased than the non-diabetic premenopausal group ($p<0.001$, $p<0.001$, and $p<0.05$, respectively) (**Table 1**).

Comparison of inflammation and endothelial function markers

Serum ET-1 levels were found significantly higher in the type 2 diabetic premenopausal group compared to the non-diabetic premenopausal group and the non-diabetic premenopausal group ($p<0.05$, and $p<0.01$, respectively). ET-1, IL-6, and YKL-40 values were increased in the type 2 diabetic premenopausal group than the non-diabetic premenopausal group, although the differences were not significant. ET-1, IL-6, and YKL-40 values were found to be significantly higher in the type 2 diabetic postmenopausal group compared with the non-diabetic postmenopausal group ($p<0.001$, $p<0.05$, $p<0.01$, respectively). ET-1, IL-6, and YKL-40 values were higher in the type 2 diabetic postmenopausal group compared with the non-diabetic premenopausal group ($p<0.01$, $p<0.05$, $p<0.01$, respectively). There were no significant differences in serum eNOS and TNF values in the comparison of the groups ($p>0.05$) (**Table 2**).

ROC curve analysis of inflammation and endothelial function markers

In the next step, we wanted to evaluate the diagnostic information of inflammation and endothelial function markers concerning menopause status and type 2 diabetes. First, the AUC values were determined for serum ET-1 (AUC=0.839, $p=0.001$) between the type 2 diabetic premenopausal vs. the non-diabetic premenopausal groups. Second, the AUC values of serum ET-1 (AUC=0.847, $p=0.001$), IL-6 (AUC=0.811, $p<0.01$), and YKL-40 (AUC=0.840, $p=0.001$) were determined between the type 2 diabetic postmenopausal vs. the non-diabetic postmenopausal groups. Then, ROC curve analysis was performed between the type 2 diabetic postmenopausal vs. the non-diabetic premenopausal groups. The AUC values were calculated for serum ET-1 (AUC=0.933, $p<0.001$), IL-6 (AUC=0.812, $p<0.01$), and YKL-40 (AUC=0.880, $p<0.001$). Finally, ROC curve analysis was performed between the type 2 diabetic postmenopausal vs. the type 2 diabetic premenopausal groups. The significance was determined in serum YKL-40 values (AUC=0.732, $p<0.05$) (Table 3). There were no statistically significant differences in the other parameters. For all testing, sensitivity and specificity values were given in **Table 3**.

Table 1. Comparison of demographic variables

	Non-diabetic Premenopausal (n:20)	Type 2 diabetic Premenopausal (n:20)	Non-diabetic Postmenopausal (n:20)	Type 2 diabetic Postmenopausal (n:20)
Age (Years)	40.12±10.78	43.86±7.35	58.29±6.42^{b***,c**}	63.60±8.14^{d***,e***}
BMI (kg/m^2)	29.86±4.89	32.36±3.92	30.90±7.07	33.24±7.49
Menopause duration (Years)	NA	NA	11.56±7.32	15.73±10.01
Diabetes duration (Years)	NA	9.45±4.67	NA	12.11±3.07
SBP (mmHg)	112.50±14.37	129.71±10.27^{a**}	127.41±19.09	139.20±25.55^{e**}
DBP (mmHg)	71.06±8.54	82.00±8.29^{a*}	74.82±13.96	78.27±8.15
Glucose (mg/dl)	87.38±6.01	115.86±22.00^{a***}	92.35±12.36^{c*}	134.40±41.70^{e***,f***}
HbA1c (%)	5.55±0.40	7.16±0.86^{a***}	5.57±0.44^{c***}	7.03±1.16^{e***,f***}
Insulin (mIU/L)	9.37±3.43	15.06±4.36^{a**}	13.04±5.91^{b*}	16.37±7.02^{e**}
Insulin resistance (mg/dl)	1.99±0.72	4.16±1.18^{a***}	3.00±1.45^{b*}	5.34±2.38^{e***,f*}

Values are given as Mean ± Standard Deviation; BMI, Body mass index; NA, not applicable; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HbA1c, Glycated haemoglobin A1c; LDL, Low density lipoprotein; HDL, High density lipoprotein; [a]Type 2 diabetic premenopausal vs. Non-diabetic premenopausal; [b]Non-diabetic postmenopausal vs. Non-diabetic premenopausal; [c]Non-diabetic postmenopausal vs. Type 2 diabetic premenopausal; [d]Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal; [e]Type 2 diabetic postmenopausal vs. Non-diabetic premenopausal; [f]Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal¸*p<0.05, **p<0.01, ***p<0.001.

Table 2. Comparison of serum proinflammatory and endothelial function parameters

	Non-diabetic Premenopausal (n:20)	Type 2 diabetic Premenopausal (n:20)	Non-diabetic Postmenopausal (n:20)	Type 2 diabetic Postmenopausal (n:20)
ET-1 (pg/ml)	0.51±0.29	0.93±0.43^{a*,b**}	0.43±0.36	0.97±0.31^{c***,d**}
eNOS (pg/ml)	0.20±0.05	0.21±0.03	0.21±0.04	0.23±0.05
IL-6 (pg/ml)	2.73±0.40	3.38±0.97	2.72±0.42	3.47±0.75^{c*,d*}
TNF-α (pg/ml)	26.22±1.57	26.18±2.03	26.11±1.60	26.46±2.94
YKL-40 (pg/ml)	1178.51±223.45	1282.43±292.74	1226.28±259.38	1483.12±194.64^{c**,d**}

Values are given as Mean ± Standard Deviation. [a]Type 2 diabetic premenopausal vs. Non-diyabetic premenopausal; [b]Non-diabetic postmenopausal vs. Type 2 diabetic premenopausal ; [c]Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal ; [d]Type 2 diabetic postmenopausal vs. Non-diabetic premenopausal ; *p<0.05, **p<0.01, ***p<0.001.

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Table 3. Diagnostic information of serum endothelial function and proinflammatory parameters

	Sensitivity	Specificity	AUC (95%CI)	p
Serum ET-1				
Type 2 diabetic premenopausal vs. Non-diabetic premenopausal	87.5%	71.4%	0.839(0.693-0.986)	0.001
Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal	76.5%	85.8%	0.847(0.707-0.987)	0.001
Type 2 diabetic postmenopausal vs. Non- diabetic premenopausal	87.5%	85.7%	0.933(0.846-0.999)	<0.001
Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal	42.9%	71.4%	0.510(0.278-0.742)	NS
Non- diabetic postmenopausal vs. Non-diabetic premenopausal	43.8%	64.7%	0.610(0.404-0.816)	NS
Serum eNOS				
Type 2 diabetic premenopausal vs. Non-diabetic premenopausal	62.5%	64.7%	0.656(0.458-0.855)	NS
Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal	41.2%	50.1%	0.519(0.304-0.734)	NS
Type 2 diabetic postmenopausal vs. Non- diabetic premenopausal	62.5%	71.4%	0.596(0.372-0.820)	NS
Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal	42.9%	50.2%	0.528(0.306-0.750)	NS
Non- diabetic postmenopausal vs. Non-diabetic premenopausal	62.5%	76.5%	0.658(0.467-0.849)	NS
Serum IL-6				
Type 2 diabetic premenopausal vs. Non-diabetic premenopausal	43.8%	78.6%	0.670(0.472-0.868)	NS
Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal	58.8%	92.9%	0.811(0.678-0.956)	<0.01
Type 2 diabetic postmenopausal vs. Non- diabetic premenopausal	56.3%	92.8%	0.812(0.660-0.965)	<0.01
Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal	57.1%	64.3%	0.607(0.373-0.841)	NS
Non- diabetic postmenopausal vs. Non-diabetic premenopausal	37.5%	64.7%	0.513(0.308-0.718)	NS
Serum TNF-α				
Type 2 diabetic premenopausal vs. Non-diabetic premenopausal	75.0%	50.0%	0.547(0.327-0.766)	NS
Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal	41.2%	64.3%	0.559(0.349-0.769)	NS
Type 2 diabetic postmenopausal vs. Non- diabetic premenopausal	50.1%	64.4%	0.592(0.382-0.801)	NS
Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal	42.9%	92.8%	0.635(0.421-0.849)	NS
Non- diabetic postmenopausal vs. Non-diabetic premenopausal	43.8%	64.7%	0.542(0.341-0.744)	NS
Serum YKL-40				
Type 2 diabetic premenopausal vs. Non-diabetic premenopausal	56.3%	57.1%	0.589(0.377-0.801)	NS
Type 2 diabetic postmenopausal vs. Non-diabetic postmenopausal	76.5%	92.9%	0.840(0.693-0.987)	0.001
Type 2 diabetic postmenopausal vs. Non- diabetic premenopausal	81.2%	92.8%	0.880(0.747-0.998)	<0.001
Type 2 diabetic postmenopausal vs. Type 2 diabetic premenopausal	64.3%	71.4%	0.732(0.534-0.930)	<0.05
Non- diabetic postmenopausal vs. Non-diabetic premenopausal	62.5%	58.8%	0.522(0.319-0.725)	NS

AUC, area under the curve; NS, not significant.

DISCUSSION

Menopause is the period, when progesterone secretion ends, the function of ovary losses, and follicles undergo atresia due to ageing . During this period, the decreasing oestrogenlevel causes dysfunction in the endothelial tissue. Type 2 diabetes mellitus is a disease, which can cause endothelial dysfunction and vascular diseases (2).

In the literature, endothelial dysfunction and inflammatory status have been evaluated with various markers in the menopausal period and type 2 diabetes mellitus (3-5) but, there are limited studies providing information about type 2 diabetes in the postmenopausal period. Therefore, in this study, endothelial function and inflammatory status were investigated by serum ET-1, eNOS, IL-6, TNF-α, and YKL-40 levels, and were evaluated the diagnostic efficacy of these markers in postmenopausal type 2 diabetes mellitus. Additionally, it was evaluated that the menopausal period and/or type 2 diabetes were more effective in endothelial dysfunction.

ET-1 is a molecule, which causes inhibition of vasoactive agent secretion from the endothelium with its proinflammatory and constrictor effects. Endothelial dysfunction develops due to ET-1 activity, and vascular complications such as nephropathy and retinopathy occur in diabetes. Impairment of vascular tissue regulation promotes the formation of atherosclerosis in patients with diabetes (6).

When we evaluated our ET-1 findings due to diabetes, a significant increase was observed in type 2 diabetic postmenopausal compared with non-diabetic postmenopausal subjects (p <0.01). Similarly, higher serum ET-1 values were obtained in patients with the type 2 diabetic premenopausal compared with the non-diabetic premenopausal group (p <0.05). (Table 2). ET-1 levels due to menopausal status showed that there was no significant difference between the non-diabetic postmenopausal and the type 2 diabetic postmenopausal, the non-diabetic premenopausal, and the type 2 diabetic premenopausal patients. The ET-1 values showed that a significant increase occurred in type 2 diabetic postmenopausal patients compared with the non-diabetic pre- and postmenopausal groups (p <0.01, and p<0.001, respectively) (Table 2).

In the literature, contradictory findings have been reported in studies examining the ET-1 levels in diabetes. Besides reports, which showed increased ET-1 levels, there were decreased ET-1 levels in other (7-11). Studies evaluating ET-1 levels in menopausal periods have reported that plasma ET-1 levels increased in postmenopausal women compared with the premenopausal group (12-14). Our findings showed that the serum ET-1 level increased in type 2 diabetes, no significant change was observed during the menopause period, but the increase was at the highest level in type 2 diabetic postmenopausal individuals.

These findings could indicate endothelial dysfunction in postmenopausal diabetes.

Endothelial dysfunction and atherosclerosis occur because of impaired compensatory mechanisms of the circulatory system. The synthesis of many molecules, including NO, was damaged during this period (15). NO is an important protective molecule in the vasculature, and the eNOS enzyme is responsible for most of the vascular NO production. NO is a potent vasodilator in vascular smooth muscle, regulates regional blood flow, and also has antithrombotic, anti-inflammatory, and antiproliferative effects. Loss of NO contributes to impaired vascular relaxation, platelet aggregation, increased proliferation of vascular smooth muscle, and leukocyte adhesion to the endothelium (16).

When our eNOS results were evaluated, there was no significant difference between eNOS levels of type 2 diabetic postmenopausal patients and non-diabetic postmenopausal individuals. Similarly, there was no significant change between eNOS levels in type 2 diabetic premenopausal patients and non-diabetic premenopausal individuals. Also, when we assessed eNOS values between the pre- and postmenopausal periods, there was no significant change (Table 2).

In the literature, studies have been conducted on NO levels in patients with diabetes, and conflicting results have been reported. Adela et al. reported that serum NO levels increased in type 2 diabetes patients compared with the control group, but other studies were reporting decreased, or not changed NO levels (17-19). Our findings show that the possible NO deficiency in type 2 diabetes and the postmenopausal period is not due to decreased eNOS levels or activity. Our serum eNOS levels may not have changed significantly, due to the lack ofvascular complications in our diabetic patients.

It has been reported that inflammation and glucose metabolism impairment affect diabetes pathogenesis (3). IL-6 is a cytokine, which contributes to inflammation, and has pleiotropic effects ranging from immune system stimulation to tissue damage (20). TNF-α, another proinflammatory cytokine, plays a role in the pathogenesis of type 2 diabetes. It is also reported that the expression of proinflammatory and prothrombotic factors might contribute to endothelial activation (21). Therefore, our study was examined serum levels of TNF-α and IL-6 in the postmenopausal period and type 2 diabetes disease.

Our findings showed elevation of serum IL-6 levels in the type 2 diabetic postmenopausal group compared with the non-diabetic postmenopausal group. Additionally, IL-6 levels were increased in type 2 diabetic postmenopausal individuals compared with non-diabetic premenopausal individuals. There was no significant difference in serum TNF-α levels related to type 2 diabetes, and the postmenopausal period (Table 2).

In the literature, many studies reported that TNF-α was increased in diabetes and postmenopausal period (5, 22, 23). Doganay et al. reported that TNF-α and IL-6 levels were higher in diabetic individuals, who developed retinopathy, compared with patients without complications, and there was no significant difference between patients without complications and healthy individuals (24).

Conflicting results were reported in studies examining IL-6 and TNF-α levels related to menopausal periods. It has been reported that IL-6 levels increased, but TNF-α values did not change (25), IL-6 levels increased and TNF-α decreased (4), TNF-α levels increased and IL-6 levels did not change in the postmenopausal period (26). Mascarenhas-Melo et al. reported an increase in TNF-α values in postmenopausal diabetic cases compared with premenopausal cases (27). In another study, no significant increase was found in TNF-α values in the postmenopausal period compared to the premenopausal period (28). Our findings showed that IL-6 is more effective than TNF-α in the proinflammatory process related to diabetes and the menopause period, and IL-6 is at the highest level in the type 2 diabetic postmenopausal group (Table 2).

Recently, YKL-40 levels have been used as inflammatory markers in different diseases. It has been shown that the YKL-40 synthesis level is an indicator of the presence and degree of inflammation, and is associated with angiogenesis and fibrosis in the healing process of damaged tissue (29). In our study, we examined whether this marker can be used for inflammatory evaluation in the postmenopausal period and in diabetes.

Our findings were showed that serum YKL-40 levels increased in the type 2 diabetic postmenopausal group compared with the non-diabetic postmenopausal group ($p < 0.01$). Additionally,YKL-40 levels of type 2 diabetic postmenopausal individuals were increased compared with the non-diabetic premenopausal individuals ($p < 0.01$).

Studies examining the YKL-40 level in diabetes reported that levels in the circulation were increased (29, 30). Kaya et al. reported that increased serum YKL-40 values were in patients with diabetic retinopathy compared with the control group (31). It was also stated that the increased YKL-40 in type 2 diabetes is associated with hyperglycemia and insulin resistance, and it can be considered an appropriate parameter for preventing endothelial dysfunctions and complications in diabetes (32).

According to our findings, we may say that serum YKL-40 increases in diabetes. Decreased estrogen activity in the postmenopausal period and inflammatory disorders on the vascular system could be an important causes of increased YKL-40 secretion in the postmenopausal type 2 diabetic subjects.

In this study, we also evaluated the diagnostic performance of endothelial function and inflammation markers by comparing type 2 diabetic and non-diabetic women in pre-and post-menopausal periods. Our results showed that ET-1, IL-6, and YKL-40 could distinguish type 2 diabetic postmenopausal and non-diabetic premenopausal cohorts (AUC: 0.933; AUC:0.812, and AUC:880, respectively). ET-1, IL-6, and YKL-40 performedsimilarly in distinguishing type 2 diabetic postmenopausal and non-diabetic postmenopausal cohorts (AUC: 0.847; AUC:0.811, and AUC:840, respectively) (Table 3).

CONCLUSION

Our findings showed that the highest serum ET-1, IL-6, and YKL-40 levels occur in type 2 diabetes, especially in the postmenopausal period. Increased levels of these parameters might indicate endothelial dysfunction and inflammation in the postmenopausal period and diabetes. Analysis of ROC curves also showed that ET-1, IL-6, and YKL-40 could distinguish type 2 diabetic postmenopausal and non-diabetic premenopausal cohorts. If these markers

are examined in type 2 diabetic postmenopausal individuals, inflammation could be detected at an early stage, and the risk of vascular complications might be reduced.

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Author Contributions: Conceived and designed the experiments: **ST, YA, and NB**. Enrolled patients: **YA and HCA**. Analyzed the data: **YA, HCA, and NB**. Wrote the first draft of the manuscript: **NB.** Contributed to the writing of the manuscript: all authors. All the authors agreed with the manuscript's results and conclusions. All the authors have read, and confirm that they meet, ICMJE criteria for authorship.

Ethical approval: This study was approved by the Ethics Committee of Istanbul University-Cerrahpasa, Cerrahpasa Medical Faculty (Approval Number: 332891/2017). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

REFERENCES

1. Özşahin AK, Asma S, Aksoyek A, Gereklioğlu Ç, Korur A. Obesity-Insulin Resistance and Diabetes. Turkish Journal of Family Medicine and Primary Care. 2015;9(2):36-9.

2. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics. 2005;115(4):e500-e3.

3. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama. 2001;286(3):327-34.

4. Cioffi M, Esposito K, Vietri MT, Gazzerro P, D'Auria A, Ardovino I, et al. Cytokine pattern in postmenopause. Maturitas. 2002;41(3):187-92.

5. July M, Faiz S, Yaqub A, Santhanam P, Douglas J, Stanek R, et al. Role of adipokines and inflammatory markers in postmenopausal hypertension. Minerva endocrinologica. 2017;43(2):101-8.

6. Kaur R, Kaur M, Singh J. Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: molecular insights and therapeutic strategies. Cardiovascular diabetology. 2018;17(1):1-17.

7. Takahashi K, Ghatei M, Lam H-C, O'halloran D, Bloom S. Elevated plasma endothelin in patients with diabetes mellitus. Diabetologia. 1990;33(5):306-10.

8. Ferri C, Pittoni V, Piccoli A, Laurenti O, Cassone MR, Bellini C, et al. Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. The Journal of Clinical Endocrinology & Metabolism. 1995;80(3):829-35.

9. Takeda Y, Miyamori I, Yoneda T, Takeda R. Production of endothelin-1 from the mesenteric arteries of streptozotocin-induced diabetic rats. Life sciences. 1991;48(26):2553-6.

10. Fernandez-Cruz A, Martin P, Fernandez L, Sanchez J, Ibarra J, Moya J, et al. Plasma endothelin is increased in young essential hypertensives but not in elderly essential or diabetic hypertensives. Journal of Hypertension. 1993;11:S146-S7.

11. Wang M, Sui J, Wang S, Wang X. Correlations of carotid intima-media thickness with endothelial function and atherosclerosis degree in patients with type 2 diabetes mellitus. Clinical hemorheology and microcirculation. 2019;72(4):431-9.

12. Wilcox JG, Hatch IE, Gentzschein E, Stanczyk FZ, Lobo RA. Endothelin levels decrease after oral and nonoral estrogen in postmenopausal women with increased cardiovascular risk factors. Fertility and sterility. 1997;67(2):273-7.

13. Vural P, Akgul C, Canbaz M. Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. Pharmacological research. 2006;54(4):298-302.

14. Khatun MT, Jesmin S, Rahman A, Ahsan HA, Islam M, Akter S, et al. Assessment of circulatory endothelin-1 level among pre-and post-menopausal rural women in Bangladesh: Result from a population-based study. Life Sciences. 2013;25(93):e68.

15. Fernández-Mejía C. Oxidative stress in diabetes mellitus and the role of vitamins with antioxidant actions. Oxidative stress and chronic degenerative diseases-a role for antioxidants. 2013;209.

16. Endres M, Laufs U, Liao JK, Moskowitz MA. Targeting eNOS for stroke protection. Trends in neurosciences. 2004;27(5):283-9.

17. Adela R, Nethi SK, Bagul PK, Barui AK, Mattapally S, Kuncha M, et al. Hyperglycaemia enhances nitric oxide production in diabetes: a study from South Indian patients. PloS one. 2015;10(4):e0125270.

18. Yenisey Ç, Öge A, Serter M, Bolaman Z. Serum interleukin-1 (IL-1beta) and nitric oxide (NO) levels in diabetes mellitus. Medical Journal of Ege University. 2001;11(1):1-5.

19. Ghosh A, Sherpa ML, Bhutia Y, Pal R, Dahal S. Serum nitric oxide status in patients with type 2 diabetes mellitus in Sikkim. International Journal of Applied and Basic Medical Research. 2011;1(1):31.

20. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. Annals of internal medicine. 1998;128(2):127-37.

21. Makino N, Maeda T, Sugano M, Satoh S, Watanabe R, Abe N. High serum TNF-α level in Type 2 diabetic patients with microangiopathy is associated with eNOS down-regulation and apoptosis in endothelial cells. Journal of Diabetes and its Complications. 2005;19(6):347-55.

22. Taleb-Belkadi O, Chaib H, Zemour L, Fatah A, Chafi B, Mekki K. Lipid profile, inflammation, and oxidative status in peri-and postmenopausal women. Gynecological Endocrinology. 2016;32(12):982-5.

23. Blank SE, Johnson EC, Weeks DK, Wysham CH. Circulating dendritic cell number and intracellular TNF-α production in women with type 2 diabetes. Acta diabetologica. 2012;49(1):25-32.

24. Doganay S, Evereklioglu C, Er H, Türköz Y, Sevinc A, Mehmet N, et al. Comparison of serum NO, TNF-α, IL-1β, sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. Eye. 2002;16(2):163-70.

25. Kim OY, Chae JS, Paik JK, Seo HS, Jang Y, Cavaillon J-M, et al. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. Age. 2012;34(2):415-25.

26. Kamada M, Irahara M, Maegawa M, Ohmoto Y, Takeji T, Yasui T, et al. Postmenopausal changes in serum cytokine levels and hormone replacement therapy. American journal of obstetrics and gynecology. 2001;184(3):309-14.

27. Mascarenhas-Melo F, Marado D, Palavra F, Sereno J, Coelho Á, Pinto R, et al. Diabetes abrogates sex differences and aggravates cardiometabolic risk in postmenopausal women. Cardiovascular diabetology. 2013;12(1):1-14.

28. Mascarenhas-Melo F, Sereno J, Teixeira-Lemos E, Ribeiro S, Rocha-Pereira P, Cotterill E, et al. Markers of increased cardiovascular risk in postmenopausal women: focus on oxidized-LDL and HDL subpopulations. Disease Markers. 2013;35.

29. Rathcke CN, Vestergaard H. YKL-40-an emerging biomarker in cardiovascular disease and diabetes. Cardiovascular diabetology. 2009;8(1):1-7.

30. Nielsen AR, Erikstrup C, Johansen JS, Fischer CP, Plomgaard P, Krogh-Madsen R, et al. Plasma YKL-40: a BMI-independent marker of type 2 diabetes. Diabetes. 2008;57(11):3078-82.

31. Kaya M, Kaya D, Idiman E, Kocak N, Ozturk T, Ayhan Z, et al. A novel biomarker in diabetic macular edema with serous retinal detachment: serum chitinase-3-like protein 1. Ophthalmologica. 2019;241(2):90-7.

32. Kumari RD, Babu FM, Mahendran BK. Human cartilage glycoprotein 39 (YKL-40): a View in type 2 diabetes mellitus. International Journal of Pharmaceutical Sciences and Research. 2015;6(11):4852.