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Evaluation of inflammation markers and their association with clinical features in patients with Metabolic Syndrome and Type 2 Diabetes Mellitus

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ABSTRACT

OBJECTIVE: *The aim of this study was to evaluate inflammation markers in patients with metabolic syndrome (MetS) and Type 2 Diabetes Mellitus (DM), and to investigate potential relationships between these parameters and other clinical and laboratory properties of patients.*

METHODS: *This study was conducted at Haseki Training and Research Hospital. The study group consisted of 60 patients diagnosed with* MetS and/or Type 2 DM *and 20 healthy individuals. MetS was diagnosed with the National Cholesterol Education Program - Adult Treatment Panel III diagnostic criteria. A broad range of parameters and clinical characteristics were measured and recorded, including vitals, anthropometric parameters, hepatosteatosis, microalbuminuria, C-reactive protein (CRP), fibrinogen, interleukinn-6 (IL-6), tumor necrosis factor-α (TNF-α), microalbuminuria in 24-hour urine, abdominal ultrasonography and routine biochemistry tests.*

RESULTS: *As anticipated, anthropometric measures, liver function tests and lipid profiles demonstrated higher levels among patients with MetS/type 2 DM compared to healthy subjects. Almost all inflammation markers were also elevated, evidencing the elevated baseline inflammatory activity among patients compared to healthy controls. The prevalence of hepatosteatosis (as a precursor to non-alcoholic fatty liver disease) was 51.7% among patients with MetS in the study group. Waist-to-hip ratio (p=0.012), ALT (p<0.001) and AST (P<0.001) values were higher in subjects with hepatosteatosis. The prevalence of microalbuminuria was 38.3% and these patients had significantly higher diastolic and systolic blood pressure values (p=0.005 and p=0.002, respectively).*

CONCLUSIONS: *In conclusion, patients with Type 2 DM and MetS have significantly elevated inflammatory markers and a high frequency of hepatosteatosis and microalbuminuria which appear to be associated with different clinical characteristics. More comprehensive prospective studies with Type 2 DM and MetS patients are needed.*

Keywords: *Humans; Cross-Sectional Studies; Metabolic Syndrome; Diabetes Mellitus, Type 2; Obesity; Insulin Resistance; Inflammation; Blood Glucose; C-Reactive Protein; Cholesterol, HDL; Cholesterol, LDL; Triglycerides; Albuminuria* /urine; Non-alcoholic Fatty Liver Disease*/ metabolism*
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INTRODUCTION

Metabolic syndrome (MetS) is a global problem that has become one of the major health hazards of the modern world (1). The definition and diagnostic criteria remain debated despite the fact that the syndrome is strongly characterized by a clustering of cardiometabolic risk factors including abdominal obesity, atherogenic

dyslipidemia, high blood pressure, prothrombotic state, chronic low-grade inflammation, insulin resistance and high blood pressure (2). In 1988, Reaven was the first to identify this concerted phenomenon (calling it syndrome X) by describing it as insulin resistance alongside a number of abnormalities which came together to increase cardiovascular risks. The qualifier "metabolic" was later added to distinguish this entity from syndrome X in cardiology(3).

In a meta-analysis including data from twenty-eight million people worldwide, the global prevalence of MetS was reported to range from 12.5–31.4% depending on the diagnostic criteria. Prevalence was significantly higher in the Eastern Mediterranean Region and the United States and also demonstrated an increase with income level (4). A meta-analysis from Turkey reported prevalence as being 38.3% in women, 26.8% in men (32.9% overall) (5). Increased consumption of high-calorie and low-fiber fast foods and decreased physical activity are important factors that elevate risks for this syndrome (1).

MetS has received considerable attention, partially owing to the importance attributed to the disease by major organizations such as the World Health Organization (WHO), the European Working Group on Insulin Resistance (EGIR) and the National Cholesterol Education Program (NCEP). Numerous topics have been discussed about MetS, its risks, and associated outcomes such cardiovascular disease and diabetes mellitus (DM). MetS is a very strong predictor of DM onset (6). The opposite is also true, type 2 DM risk decreases with improvements in MetS components, as determined by a nationwide cohort in South Korea (7). The growing burden of Type 2 DM continues to be a major concern in healthcare worldwide. The proportion of people affected by Type 2 DM was approximately 6059 cases per 100,000 people in 2017 (6.28% of the world's population, 462 million people) and the global prevalence of Type 2 DM is projected to increase to 7079 cases per 100,000 people by 2030, with a continuing upward trend worldwide (8). Since Type 2 DM is a major risk factor for cardiovascular complications and mortality in MetS patients, the association of MetS and Type 2 DM is a topic that necessitates constant attention from the medical perspective. When discussing the links between MetS and Type 2 DM, it is important to remember that both of these diseases have a causal relationship with inflammation (9).

Metabolic syndrome-associated inflammation is characterized by a chronic increase in the serum concentration of proinflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (10). Based on the association between the concentration of inflammatory markers and the components of the metabolic syndrome, inflammation is presumed to be an event that may precede the development of metabolic disorders (11,12). The precursor of inflammation, which is thought to precede the development of MetS, is the damaged and altered endothelium (13). In this mechanism, chronic overload of free fatty acids and glucose, which can trigger inflammatory pathways directly or through increased reactive oxygen species production (14) and lead to endothelial stress and increased platelet activity, is probably the first blow (9). In a prospective study evaluating the results obtained after six years of follow-up, hs-CRP and IL-6 levels were reported to be reliable predictors of the incidence and

persistence of MetS (15). Since low-grade chronic inflammation may increase the risk of metabolic syndrome and diabetes (16), it is important to evaluate the relationship between MetS, Type 2 DM, clinical outcomes, and inflammation. Therefore, in this study, we aimed to evaluate inflammation markers in patients with MetS and Type 2 DM and to assess and discuss the relationships between these parameters and other clinical/laboratory features (such as hepatosteatosis and microalbuminuria) of patients with MetS and/or DM.

METHODS

Design and participants

This study was conducted at Haseki Training and Research Hospital with the inclusion of patients with MetS or type 2 DM as the primary subgroup. The study was approved by the local ethics committee **(**Ethical approve was taken from ethical board. of Haseki Hospital (16/23.05.2024)**)** and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Sixty patients diagnosed with MetS and/or Type 2 DM and 20 healthy individuals admitted to Internal Medicine and Endocrinology outpatient clinics were examined. Patients with a diagnosis of obesity who were on pharmacologic or dietary treatment for obesity were not included in the study group. In diabetic patients, those with an HbA1c value of at least 6.5% who received non-insulin oral antidiabetic treatment were included in the study group.

Diagnostic definitions

National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATP III) diagnostic criteria were used for the diagnosis of MetS (waist circumference: male >102 cm, female >88 cm, triglycerides >150 mg/dL, high-density lipoprotein (HDL): male <40 mg/dL, female <50 mg/dL, blood pressure ≥130/85 mmHg, fasting plasma glucose ≥100 mg/dL). MetS was considered to be present if three or more of the criteria were present (17). The American Diabetes Association (ADA) diagnostic criteria were used for the diagnosis of Type 2 DM (HbA1C ≥6.5%, fasting blood glucose (FBG) ≥126 mg/dL, 2-hour plasma glucose ≥200 mg/dL during oral glucose tolerance test (OGTT), or a random plasma glucose level of ≥200 mg/dL) (18).

Parameters and measurements

Laboratory data

Samples required for the measurement of all examined parameters were obtained following inclusion into the study. Abdominal ultrasonography and biochemistry analyses were performed in a routine manner and the results were recorded for analyses. In the abdominal ultrasonography examination, the anteroposterior length of the liver in the midclavicular line was measured using a Digital Sonography 5500 brand device and a 3.5 mHz convex probe. Patients with a minimally diffuse increase in hepatic echogenicity were considered to have hepatosteatosis, given that the edges of the intrahepatic vessels and the diaphragm were normal. We considered hepatosteatosis to be a precursor finding of non-alcoholic fatty liver disease (NAFLD).

In all subjects, basic lipid profile parameters [HDL, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL)], C-reactive protein (CRP), fibrinogen, sedimentation, insulin and glucose levels were measured in venous blood samples after a 12-hour fast. Insulin, C-peptide and TNF-α were measured via a radioimmunoassay method, IL-6 was measured by enzyme-linked immunosorbent assays (ELISAs),

glucose and lipids by enzymatic and colorimetric methods, and CRP and fibrinogen were quantified with a nephelometric method. Classical liver function tests were performed as part of routine examination, using calibrated devices in the routine biochemistry laboratory. Microalbuminuria was measured in 24-hour urine. Albumin/creatinine ratio values between 30 and 300 mg/g were defined as being indicative of microalbuminuria.

To measure insulin resistance, the Homeostatic Model Assessment of insulin resistance (HOMA-IR) was calculated as follows: fasting insulin level (mIU / ml) × fasting blood sugar (mmol/L) / 22.5 was used.

Vitals and anthropometric data

Diastolic and systolic blood pressure values (DBP and SBP) were following inclusion into the study. Body weight was measured in kg with room clothes and height was measured in cm with standardized measuring instruments. It was calculated with the formula body mass index (BMI) = body weight (kg) / height-squared (m2). Those with BMI values higher than 30 kg/m2 were considered to be obese and those with BMI values below 25 kg/m2 were considered normal weight. Waist circumference (cm) and hip circumference (cm) were measured and waist-hip ratio (WHR) was calculated using the formula WHR = waist circumference (cm) / hip circumference (cm).

Statistical Analysis

IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses, and the tests were considered to be significant if p values were less than 0.05 (5% alpha error). Descriptive statistics were presented by using mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables. Between-groups analyses were performed by using the Student t-test for continuous variables and by using appropriate chi-square tests for categorical variables.

RESULTS

There were 50 (62.5%) females and 30 (37.5%) males in the study and the groups were similar in terms of sex distribution ($p = 0.286$); however, the MetS group was significantly older higher compared to the control group ($p<0.001$). As anticipated, patients with MetS had significantly higher DBP ($p=0.010$), SBP ($p<0.001$), weight ($p<0.001$), and BMI ($p<0.001$) values. Again, as expected in patients with metabolic syndrome, we detected significantly higher levels of glucose ($p<0.001$), uric acid ($p<0.001$), alanine aminotransferase (ALT, $p<0.001$), aspartate aminotransferase (AST, $p=0.001$), total cholesterol ($p<0.001$), triglyceride ($p<0.001$) values in patients with MetS compared to healthy subjects. Among the inflammation markers, C-peptide ($p<0.001$), insulin ($p<0.001$), HOMA ($p<0.001$), sedimentation ($p<0.001$), CRP ($p<0.001$), white blood cell count (WBC, $p<0.001$), fibrinogen ($p<0.001$), microalbumin ($p<0.001$), IL-6 ($p<0.001$) values were significantly higher in the MetS group than in the controls (Figure 1). Of note, TNF-α was found to be significantly higher in the healthy control group (Figure 2, Table 1).

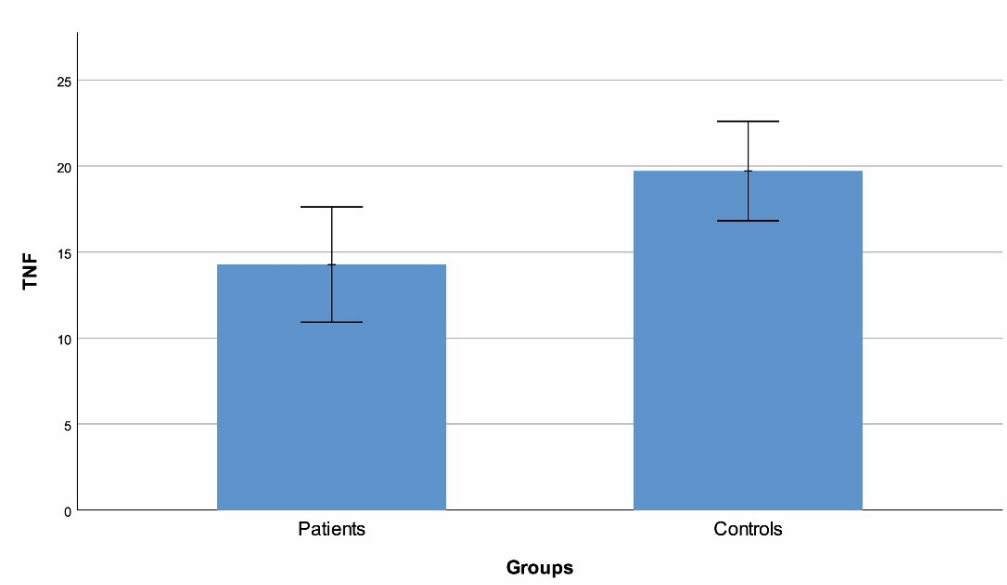

Figure 2. Tumor necrosis factor-α (TNF-α) levels (mean ± standard deviation) with regard to groups

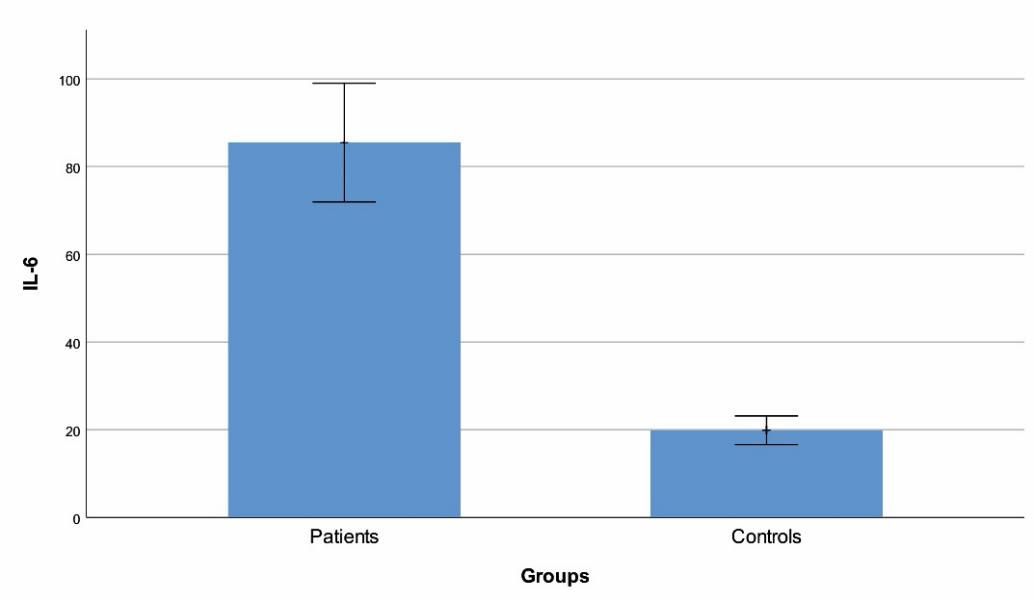

Figure 1. Interleukin-6 (IL-6) levels (mean ± standard deviation) with regard to groups

Table 1. Comparison of primary groups in terms of all examined parameters

	Groups		
	Patients (n=60)	Controls (n=20)	p
Age	56.58 ± 8.72	36.20 ± 11.33	**<0.001**
Sex			
Female	40 (66.7%)	10 (50.0%)	0.286
Male	20 (33.3%)	10 (50.0%)	
Diastolic blood pressure	94.00 ± 19.06	81.75 ± 14.08	**0.010**
Systolic blood pressure	157.08 ± 28.65	125.00 ± 17.32	**<0.001**
Weight, kg	83.98 ± 8.56	59.95 ± 9.47	**<0.001**
Height, cm	163.65 ± 7.12	174.75 ± 8.10	**<0.001**
Body mass index, kg/m^2	31.49 ± 3.94	19.66 ± 2.96	**<0.001**
Waist circumference, cm	105.28 ± 6.39	-	-
Waist-to-hip ratio	0.88 ± 0.03	-	-
Glucose	221.30 ± 59.70	84.15 ± 9.42	**<0.001**
BUN	18.23 ± 6.07	15.25 ± 1.68	**0.034**
Creatinine	1.15 ± 1.72	0.99 ± 0.33	0.682
Uric acid	4.72 ± 1.82	2.38 ± 0.35	**<0.001**
ALT	36.38 ± 9.29	25.30 ± 5.89	**<0.001**
AST	29.78 ± 11.20	20.45 ± 6.02	**0.001**
LDH	333.07 ± 66.42	299.50 ± 52.72	**0.044**
ALP	239.93 ± 65.71	146.10 ± 13.67	**<0.001**
GGT	28.62 ± 8.25	34.30 ± 10.38	**0.015**
Sodium	138.88 ± 4.58	136.90 ± 1.62	0.063
Potassium	4.68 ± 0.45	4.90 ± 0.54	0.076
Calcium	8.40 ± 0.62	8.13 ± 0.68	0.104
Phosphor	3.62 ± 0.30	3.62 ± 0.17	0.977
Total protein	7.63 ± 1.28	7.30 ± 0.99	0.296
Albumin	5.17 ± 4.53	4.29 ± 1.00	0.393
Total cholesterol	219.80 ± 45.90	114.25 ± 6.10	**<0.001**
LDL	133.83 ± 38.59	85.05 ± 4.12	**<0.001**
HDL	43.92 ± 8.40	40.05 ± 4.55	0.053
VLDL	40.73 ± 17.68	19.65 ± 3.63	**<0.001**
Triglyceride	240.97 ± 168.41	98.90 ± 17.65	**<0.001**
C-peptide	0.31 ± 0.04	0.17 ± 0.03	**<0.001**
Insulin	17.08 ± 2.90	10.45 ± 1.85	**<0.001**
HOMA	9.43 ± 3.27	2.17 ± 0.47	**<0.001**
QUICKI	0.28 ± 0.01	0.34 ± 0.01	**<0.001**
Glucose to insulin ratio	13.19 ± 3.70	8.31 ± 1.92	**<0.001**
Sedimentation	39.78 ± 6.83	18.05 ± 4.79	**<0.001**
CRP	21.47 ± 7.48	4.45 ± 2.37	**<0.001**
Hematocrit	39.25 ± 5.40	40.15 ± 4.98	0.513
Hemoglobin	12.90 ± 1.07	13.14 ± 1.33	0.417
WBC (x10^3)	9.55 ± 3.25	6.05 ± 1.11	**<0.001**
Platelet (x10^3)	276.13 ± 131.74	244.35 ± 210.65	0.429
Fibrinogen	552.18 ± 73.28	371.15 ± 105.84	**<0.001**
Microalbumin	28.70 ± 15.41	12.35 ± 10.54	**<0.001**
TNF-α	14.27 ± 3.35	19.72 ± 2.89	**<0.001**
IL-6	85.44 ± 13.54	19.87 ± 3.25	**<0.001**

Descriptive statistics were presented by using mean ± standard deviation for continuous variables and frequency (percentage) for categorical variables.

BUN: blood urea nitrogen, ALT: Alanine transaminase, AST: aspartate aminotransferase, LDH: Lactate Dehydrogenase, ALP: alkaline phosphatase, GGT: Gamma-glutamyl transferase, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, HOMA: Homeostatic Model Assessment, QUICKI: Quantitative insulin sensitivity check index, CRP:C-reactive protein, WBC: White blood cells, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6

The frequency of hepatosteatosis among patients with MetS in the study group was 51.7%. Among patients diagnosed with MetS and Type 2 DM, waist-to-hip ratio (p=0.012), ALT (p<0.001) and AST (p<0.001) values were found to be higher in those with hepatosteatosis. No significant difference was found in terms of inflammatory markers between those with and without hepatosteatosis (Table 2).

Table 2. Patient characteristics and laboratory measurements with regard to hepatosteatosis subgroups

	Hepatosteatosis		
	No (n=29)	Yes (n=31)	p
Body mass index, kg/m^2	31.59 ± 3.52	31.39 ± 4.35	0.846
Waist circumference, cm	105.10 ± 5.64	105.45 ± 7.12	0.834
Waist-to-hip ratio	0.87 ± 0.03	0.89 ± 0.03	**0.012**
Glucose	210.83 ± 63.51	231.10 ± 55.13	0.191
Uric acid	5.65 ± 5.93	4.86 ± 1.82	0.482
ALT	28.48 ± 6.92	43.77 ± 2.86	**<0.001**
AST	19.86 ± 6.95	39.06 ± 4.19	**<0.001**
LDH	320.69 ± 74.13	344.65 ± 57.11	0.165
ALP	234.79 ± 62.86	244.74 ± 68.94	0.562
GGT	29.59 ± 6.45	27.71 ± 9.66	0.382
Total cholesterol	221.28 ± 51.83	218.42 ± 40.38	0.812
LDL	133.14 ± 41.63	134.48 ± 36.20	0.894
HDL	42.38 ± 7.34	45.35 ± 9.17	0.173
VLDL	47.10 ± 19.95	34.77 ± 12.95	**0.006**
Triglyceride	253.66 ± 153.55	229.10 ± 182.96	0.577
Insulin	16.86 ± 2.96	17.29 ± 2.88	0.571
HOMA	8.96 ± 3.66	9.87 ± 2.84	0.285
QUICKI	0.28 ± 0.01	0.28 ± 0.01	1.000
Glucose to insulin ratio	12.62 ± 3.16	13.73 ± 4.13	0.249
Sedimentation	41.55 ± 7.12	38.13 ± 6.21	0.052
CRP	22.38 ± 7.06	20.61 ± 7.87	0.364
WBC (x10^3)	10.02 ± 3.65	9.10 ± 2.81	0.277
Platelet (x10^3)	291.38 ± 134.16	261.87 ± 130.00	0.390
Fibrinogen	549.90 ± 51.99	554.32 ± 89.61	0.818
Microalbumin	29.59 ± 16.81	27.87 ± 14.21	0.670
TNF-α	14.95 ± 3.25	13.64 ± 3.37	0.131
IL-6	86.17 ± 14.79	84.77 ± 12.46	0.692

Descriptive statistics were presented by using mean ± standard deviation.
ALT: Alanine transaminase, AST: aspartate aminotransferase, LDH: Lactate Dehydrogenase, ALP: alkaline phosphatase, GGT: Gamma-glutamyl transferase, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, HOMA: Homeostatic Model Assessment,

QUICKI: Quantitative insulin sensitivity check index, CRP:C-reactive protein, WBC: White blood cells, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6

The frequency of patients with microalbuminuria was 38.3%. DBP (p=0.005) and SBP values (p=0.002) were significantly higher in MetS patients with microalbuminuria. In addition, patients with microalbuminuria had lower platelet (p=0.005), TNF-α (p=0.002) and IL-6 (p=0.049) levels (Table 3).

Table 3. Comparisons with regard to microalbuminuria subgroups

	Microalbuminuria		
	No (n=37)	Yes (n=23)	p
Age	55.97 ± 8.43	57.57 ± 9.28	0.494
Diastolic blood pressure	88.65 ± 13.83	102.61 ± 23.15	**0.005**
Systolic blood pressure	148.24 ± 18.23	171.30 ± 36.25	**0.002**
Body mass index, kg/m^2	31.01 ± 4.07	32.26 ± 3.68	0.235
Waist circumference, cm	104.68 ± 5.51	106.26 ± 7.64	0.356
Waist-to-hip ratio	0.89 ± 0.03	0.88 ± 0.03	0.214
Glucose	210.32 ± 53.54	238.96 ± 65.88	0.070
BUN	19.03 ± 6.46	16.96 ± 5.25	0.201
Creatinine	1.39 ± 2.16	0.76 ± 0.21	0.170
LDH	319.19 ± 62.21	355.39 ± 68.23	**0.039**
Total protein	7.78 ± 1.50	7.39 ± 0.77	0.253
Albumin	4.75 ± 1.03	5.86 ± 7.25	0.361
Total cholesterol	228.57 ± 47.44	205.70 ± 40.36	0.060
LDL	141.00 ± 39.81	122.30 ± 34.28	0.068
HDL	44.70 ± 8.38	42.65 ± 8.47	0.363
VLDL	39.89 ± 16.46	42.09 ± 19.80	0.643
Triglyceride	263.38 ± 197.00	204.91 ± 101.73	0.193
C-peptide	0.31 ± 0.04	0.31 ± 0.05	0.917
Insulin	16.89 ± 2.89	17.39 ± 2.95	0.521
HOMA	8.89 ± 3.08	10.29 ± 3.44	0.107
QUICKI	0.28 ± 0.01	0.28 ± 0.01	0.085
Glucose to insulin ratio	12.64 ± 3.19	14.08 ± 4.34	0.145
Sedimentation	40.35 ± 6.70	38.87 ± 7.09	0.419
CRP	21.70 ± 7.22	21.09 ± 8.03	0.762
Platelet (x10^3)	313.22 ± 143.43	216.47 ± 82.74	**0.005**
Fibrinogen	557.70 ± 72.01	543.30 ± 76.05	0.464
TNF-α	15.27 ± 3.22	12.65 ± 2.95	**0.002**
IL-6	88.13 ± 12.89	81.12 ± 13.71	**0.049**

Descriptive statistics were presented by using mean ± standard deviation.
BUN: blood urea nitrogen, ALT: Alanine transaminase, AST: aspartate aminotransferase, LDH: Lactate Dehydrogenase, ALP: alkaline phosphatase, GGT: Gamma-glutamyl transferase, HDL: High-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein, HOMA: Homeostatic Model Assessment, QUICKI: Quantitative insulin sensitivity check index, CRP:C-reactive protein, WBC: White blood cells, TNF-α: Tumor necrosis factor-α, IL-6: Interleukin-6

DISCUSSION

Regardless of its origin, chronic low-grade inflammation accompanying metabolic syndrome has an important role in both the formation of MetS and its consequences such as Type 2 DM and cardiovascular disease (19). In this study, we evaluated Type 2 DM and MetS patients in terms of inflammation markers and highlighted potential relationships with hepatosteatosis and microalbumiuria.

A study by Choi et al reported that metabolic syndrome was associated with sex, age, waist circumference, SBP, HDL, triglyceride, FBG and IL-10 in multiple logistic regression analysis (20). In the study of Li et al., it was reported that age, BMI, SBP, DBP, FPG, triglyceride and LDL values were found to be higher in those with a MetS diagnosis than in those without. In a study by Lee and colleagues, it was reported that age, waist circumference, BMI, SBP, DBP, FBG and triglyceride values were found to be significantly higher in people diagnosed with MetS in both men and women (21). In another study, it was reported that BMI, SBP, DBP, waist circumference, FBG and triglyceride values were found to be higher in patients with MetS (22). In the present study, consistent with the literature, DBP, SBP, weight, height, BMI, glucose, triglyceride, blood urea nitrogen (BUN), uric acid, ALT, AST, Lactate Dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol, LDL, VLDL, values were significantly higher in patients diagnosed with Type 2DM and MetS than in controls.

Various pathogenic pathways that contribute to the development of MetS result in a pro-inflammatory state, which explains the elevation of inflammatory markers such as IL-6, CRP, fibrinogen, TNF-α, etc. (23,24). Inflammation in metabolic syndrome is often referred to as “chronic low-grade” inflammation due to persistent but minor-to-moderate activation. This inflammatory state is also referred to as “metaflammation” (standing for metabolically-triggered inflammation) or “parainflammation”, a term used to describe an intermediate state between baseline and overt inflammation. Since the inflammatory landscape in MetS is not accompanied by infection, autoimmunity or tissue damage, the inflammatory activity is considered to be a unique process that has not been fully elucidated (25). One of the mechanisms proposed to explain the link between inflammation and MetS is that these cytokines are released into the circulation by adipose tissue, which leads to hepatic CRP stimulation (24). In the study of Choi et al., it was reported that adiponectin and IL-10 levels were found to be lower in people with metabolic syndrome, while serum hs-CRP and IL-6 levels were higher. Their multivariable regression analysis revealed that metabolic syndrome was independently associated with IL-10 level (20). This cytokine-based hypothesis is supported by other studies which have shown that circulating leukocyte, neutrophil, lymphocyte, basophil, monocyte, malondialdehyde, TNF-α and IL-6 levels are higher in MetS patients than in those without MetS (26). However, it also appears that these relationships are mediated by sex. In the Mexico City Diabetes Study which involved 6 years of follow-up, baseline hs-CRP was associated with the development of MetS in women but not in men (27). Furthermore, coexisting abnormalities also impact inflammatory activation, as demonstrated by Garg et al. who found that inflammatory markers (including hs-CRP and fibrinogen levels) increased with the number of metabolic abnormalities in people with MetS (28).

Furthermore, the same study reported that hs-CRP and fibrinogen were positively correlated with BMI, body fat mass, body fat percentage, and HOMA-IR. The analyses also revealed that waist-to-hip ratio was an independent determinant of hs-CRP and fibrinogen, while hs-CRP level was an accurate predictor of MetS (71% sensitivity, 78% specificity) (28). These findings indicate a stepwise progression of the disease in which multiple factors act in concert to increase inflammatory activity (and possibly vice-versa), which seems to be a central phenomenon facilitating the development of further abnormalities. In a comprehensive study examining a battery of inflammatory markers, Ingelsson and colleagues reported that among the 17 inflammatory biomarkers examined, vascular cell adhesion molecule 1 (VCAM-1), E-selectin and CRP were the markers that had the strongest relationships with MetS and insulin resistance (29). There are other studies reporting consistent associations between CRP concentrations and metabolic syndrome (20,30).

In the Framingham Heart Study, MetS was closely associated with inflammatory biomarkers in univariate analyses. However, after adjusting for confounders and covariates, the association with most inflammatory markers was reported to disappear (except for P-selectin) (31). In the current study, the inflammation markers C-peptide, insulin, HOMA, Sedimentation, CRP, WBC, Fibrinogen, Microalbumin, and IL-6 were significantly higher in patients diagnosed with MetS and Type 2 DM compared to healthy controls, while TNF-α was an outlier showing an opposite relationship. According to our results, inflammatory markers in Type 2 DM and MetS patients were significantly higher than in healthy individuals. It may be useful to evaluate these markers through longitudinal studies that follow patients with high likelihood for MetS, which can create data that could facilitate the early detection of MetS. As a matter of fact, many studies have suggested that this approach could be beneficial. In one such study, it was reported that CRP levels were higher in MetS patients with central obesity and insulin resistance than in those without, while the number of MetS criteria met by patients was found to positively correlate with CRP and ferritin levels (32). In another study, oxidative stress and plasma inflammation biomarkers (oxidized low-density lipoprotein, CRP, TNF-α, IL-6 and IL-18) were found to be higher in MetS patients diagnosed with obesity than in normal weight controls (33). In the study of Ryu et al., it was reported that an increase in WBC and hs-CRP and a decrease in adiponectin levels were observed in patients with MetS and that these parameters were correlated with the presence of MetS components (30). A study evaluating patients with MetS and obesity reported that weight loss was associated with not only improved insulin sensitivity, but also a significant decrease in inflammatory markers (25). This relationship has been solidified by the findings of a meta-analysis which reported that TNF-α, CRP, IL-8 and IL-10 levels improved in patients with MetS who received exercise intervention (34).

The links between NAFLD, MetS and Type 2 DM are additional findings that indicate a complex, multifactorial disease. Accurate understanding of these connections and early diagnosis and monitoring of existing conditions that could be indicative of progression (such as hepatosteatosis) are important for effective targeted therapies (35). NAFLD is considered the hepatic manifestation of MetS. However, the liver is not only a passive end-organ being effected by

MetS, but is actively involed in the pathogenesis and complications of metabolic syndrome (36). Approximately 90% of NAFLD patients have more than one MetS feature and approximately 33% meet three or more MetS criteria. Lipotoxicity leads to the accumulation of triglycerides in the liver as a result of the imbalance between the uptake, synthesis, export and oxidation of fatty acids and plays a dominant role in the pathophysiology of both entities (37). According to a previous population-based study, NAFLD is detected in 30% of the general adult population, which increases to 60-70% among the obese and diabetic (38). In a study conducted by Yang et al. in patients with MetS, linear relationships were found between the severity of NAFLD and waist circumference, FBG, HOMA-IR, triglycerides, HDL-C and blood pressure and after adjusting for BMI and HOMA-IR, MetS It has been reported that the probability is 3.64 times higher in those with moderate-severe NAFLD than in those with mild NAFLD (39). In a remarkable similarity with other components, MetS diagnosis and the number of criteria fulfilled by patients were found to be associated with the risk of developing NAFLD. Even in those with only one component of MetS, the risk was reported to reach around 3.6 folds of the risk in patients who did not meet any MetS criteria (40). In the present study, hepatosteatosis was present in 51.7% of our patient group. Among patients diagnosed with MetS and Type 2 DM, waist-to-hip ratio, ALT and AST values were found to be higher in those with hepatosteatosis. However, interestingly, there was no significant difference in inflammatory markers between those with and without hepatosteatosis. Based on these results, it can be said that inflammation markers may not be a clue in predicting hepatosteatosis in MetS and Type 2 DM patients, but close monitoring of waist-to-hip ratio, ALT and AST values among such patients may be useful to plan for interventions to prevent NAFLD. This is an important finding that can be employed to stratify different risks and underlying factors among patients with MetS-related laboratory findings. Effective management of NAFLD associated with MetS and Type 2 DM includes early diagnosis and optimal treatment (35). In fact, timely and correct treatment of NAFLD and MetS may also contribute to the prevention of Type 2 DM. For instance, in the presence of Type 2 DM, the emergence or progression of NAFLD can be prevented with new antihyperglycemic drug classes (SGLT2i, DPPi, GLP1-RA, etc.) combined with insulin sensitizers (metformin and pioglitazone). If MetS, NAFLD and Type 2 DM are already present in the same patient (metabolic triad), concurrent treatments can reduce cardiovascular morbidity and mortality. As such, it is evident that the treatment of the primary presenting entity of the metabolic triad significantly determines the emergence and treatment of the others (35) and, based on our data, it appears that the laboratory characteristics of patients can be used to determine management approaches among individuals suffering from MetS and/or its clinically-relevant outcomes.

Microalbuminuria, which means abnormally increased urinary albumin excretion rate in the range of 30-299 mg/g, is a sign of endothelial dysfunction and an increased risk of cardiovascular morbidity and mortality, especially in high-risk populations with metabolic comorbidities (41). Microalbuminuria in MetS is a risk factor for chronic kidney disease, even in the early stages of disease (21). It has been reported that microalbuminuria causes a similar increased risk of cardiovascular disease and even death, when compared to MetS (42). A meta-analysis evaluating a total of 57 studies with a cumulative analysis

of 10,603,067 people reported that MetS contributed to higher risks of proteinuria and albuminuria, independent of DM (43). In the study of Hao et al., it was reported that the age- and sex-adjusted risk of microalbuminuria was 1.99-fold higher in people with MetS compared to those without (44). Similar results have been described by a number of researchers (21,22,45). In this study, microalbumin value was higher in MetS patients than in healthy individuals. Confirmation of these data in future studies could potentially lead to the consideration of microalbuminuria as a primary component of MetS, and this approach could be beneficial to prevent progression to chronic kidney diseases. We believe this to be an important topic since we found the frequency of microalbuminuria to be 38.3% in our cohort. This is a markedly high value compared to the frequency of microalbuminuria reported in prior research (around 10-15%) (44,46). We think that the higher frequency we found in this study is due to the evaluation of patients diagnosed with Type 2 DM and MetS.

Microalbuminuria is also strongly associated with components of MetS. Supporting the trend of data showing stepwise progressive properties for MetS, Choi et al reported a significant relationship between the number of MetS components and the prevalence of microalbuminuria (47). In fact, the probability of Type 2 DM, MetS, hypertension and hyperglycemia have been found to be significantly elevated among people with high microalbumin concentrations (46). These overt findings are also supported by associations with biochemical and systemic parameters, including obesity, impaired FBG, high blood pressure and hypertriglyceridemia, which were correlated with proteinuria and albuminuria (43). In another study, multiple logistic regression analysis reported that high glucose, high blood pressure and obesity were independently associated with microalbuminuria (44). In the population-based study by Palaniappan et al., hypertension showed the strongest association with microalbuminuria in both males and females after adjusting for other components of MetS (48). Similarly, in the study of Lee et al., the frequency of microalbuminuria was reported to be significantly higher in those with SBP/DBP values exceeding 130/85 (21). Similar to our results, SBP was found to be higher in MetS patients with microalbuminuria compared to those without microalbuminuria (49). This relationship is supported by data showing significant correlations between microalbuminuria and MetS-related characteristics, with the strongest association being with high blood pressure (21). Xuke et al. have shown that, in MetS cases, the risk of microalbuminuria increases in parallel with the number of MetS components – even after adjusting for critical parameters like central obesity, high blood pressure, high fasting glucose and related factors (45). In this study, consistent with the results in the literature, DBP and SBP values of our patients with microalbuminuria were significantly higher.

In metabolic syndrome, chronic low-grade inflammatory states are often accompanied by metabolic changes such as DM, hypertension and obesity that are directly related to the incidence of cardiovascular disease (10). Considering that cardiovascular diseases are one of the leading causes of morbidity and mortality worldwide, treatment of MetS has an important role in reducing the heavy burden of the disease (3). It is clear that the current trend is not sustainable unless a magic cure for MetS

is introduced (unlikely) or global/political/societal concerted efforts for lifestyle changes take root. While there are some elements of MetS causation that cannot be changed at all, many can be corrected and reduced (1). The general goals of treatment for MetS are lifestyle changes, including weight loss and exercise, as well as appropriate pharmacological treatments to prevent cardiological events and DM, if not already available (24). However, the fact that the MetS diagnostic criteria used in each study are not the same may have caused differences in results between studies. There is a need to determine effective diagnostic criteria for the diagnosis of MetS, which has not yet been standardized and to achieve a global consensus on these criteria for the prevention, early diagnosis and effective treatment of MetS.

Limitations

The primary limitation of this study is its cross-sectional design. A prospective longitudinal study could provide stronger evidence to elucidate the chronology and causality between MetS, Type 2 DM, inflammation, hepatosteatosis and microalbuminuria. Another limitation is that the study was single-center and was conducted with a small number of patients. The fact that the participants in this study were selected from people applying to a health institution may have created selection bias and prevented the generalizability of the results to the society. Another limitation is that the patients in the MetS group were not similar to the control group in terms of age. The fact that the disease duration of the patients selected in the study was not standard and no evaluation was made on this subject is another limitation that may have affected our results. Despite these, this study is remarkable in that it evaluated in detail the results of patients diagnosed with Type 2 DM and MetS in terms of inflammatory markers, and examined underlying relationships with particularly hepatosteatosis and microalbuminuria.

CONCLUSIONS

Our data show that the majority of inflammatory markers are significantly increased in MetS and Type 2 DM, which were found to be especially associated with the presence of hepatosteatosis and microalbuminuria, indicating a role for these features in patient management and risk stratification. In these patients, waist-to-hip ratio, ALT, and AST can be clinically guiding in terms of the development of hepatosteatosis, while DBP and SBP appear to be primarily associated with the development of microalbuminuria. These results need to be tested in population-based and preferably longitudinal comprehensive prospective studies.

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