

The relationship of circulating MOTS-c level with liver fibrosis and metabolic components in patients with metabolic dysfunction-associated fatty liver disease

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Abstract. – OBJECTIVE: Mitochondrial open reading frame of the 12s ribosomal RNA type-c (MOTS-c) is a novel identified mitochondrial signal transmission peptide that plays an important role in glucose, amino acid and lipid metabolism. In this study, we aimed to investigate the relationship of circulating MOTS-c level with noninvasive scores of fibrosis and the components of metabolic syndrome (MetS) in patients with metabolic dysfunction-associated fatty liver disease (MAFLD).

PATIENTS AND METHODS: This was a single-center cross-sectional study, and the participants were divided into two groups based on their liver ultrasound results: the fatty liver group and the healthy control group. The MOTS-c level was measured by the ELISA method. Non-alcoholic fatty liver disease fibrosis score (NFS) and fibrosis 4 (FIB-4) were used to determine the level of liver fibrosis. Statistical analyses were performed using Statistical Package for Social Science 15.0 package program.

RESULTS: One hundred fifty patients (male, n=57) with MAFLD [median age 41.0 (14) years] and 84 healthy controls (male, n=34) [median age 36.0 (22) years] were included in this study. Patients with MAFLD had significantly lower MOTS-c levels than the healthy controls ($p=0.009$). The MOTS-c level was significantly lower in subjects with MetS (n=48) compared to those without MetS (n=186) ($p=0.01$). In the total population (n=234), MOTS-c levels negatively correlated with the presence of MAFLD, NFS, FIB-4, and components of MetS.

CONCLUSIONS: Individuals diagnosed with MetS and MAFLD tend to have lower levels of MOTS-c. Additionally, these lower levels are inversely correlated with both the components of MetS and noninvasive fibrosis scores. MAFLD negatively correlated to the MetS components and noninvasive scores of fibrosis.

Key Words:

MOTS-c, MAFLD, Metabolic syndrome, Liver fibrosis.

Introduction

Nonalcoholic fatty liver disease (NAFLD), which occurs as a result of fat accumulation in the liver, is one of the most common chronic metabolic diseases all over the world¹. The liver fat accumulation that starts because of impaired glucose and lipid metabolism in NAFLD may progress to steatohepatitis and fibrosis over the years². The terminology of NAFLD, which is used today to define subjects without chronic alcohol use, is insufficient for the correct classification of patients due to the prevalence of mild to moderate alcohol use and the increase in metabolic disorders³. In this regard, a new definition is suggested to entitle metabolic dysfunction-associated fatty liver disease (MAFLD) instead of NAFLD⁴. The most important benefit of this new nomenclature is the

clarification of the impact of metabolic dysfunction and related diseases, which play an important role in the pathophysiology of this clinically relevant condition⁵.

Mitochondria is a semi-autonomous intracellular organelle that is involved in important intracellular reactions, especially in reactive oxygen species metabolism and energy production⁶. In recent years the knowledge of mitochondria has been growing as we discover the mitochondrially derived peptides as the novel regulators of metabolism^{7,8}. The peptide products of the organelle, such as the mitochondrial open reading frame of the 12s ribosomal RNA type-c (MOTS-c), are related to glucose, lipid, and amino acid metabolism^{6,9}. MOTS-c is reported to increase glucose utilization in skeletal muscle through the activation of adenosine 5'-monophosphate-activated protein kinase-dependent mechanisms and improve insulin resistance (IR)^{6,10}. In animal studies¹¹⁻¹³, it was obtained that MOTS-c treatment improves inflammation, endothelial dysfunction, glucose, and lipid metabolism and reduces liver fat accumulation.

Although the accumulating evidence indicates that MOTS-c may play a role in metabolic functions and the pathogenesis of MAFLD, there is so far no published data about the role of circulating MOTS-c in subjects with MAFLD. Additionally, the published human reports about the relationship between MOTS-c levels and the components of metabolic syndrome (MetS) are controversial^{10,14}. In this cross-sectional study, we compared the circulating MOTS-c levels between patients with MAFLD and healthy control subjects. Also, we searched for any relationship between the MOTS-c levels and the noninvasive scores of fibrosis in patients with MAFLD. Finally, we investigated the relationship of the circulating MOTS-c levels with the components of MetS.

Patients and Methods

This single-center cross-sectional study was performed on adult patients who applied to the internal medicine outpatient clinics for routine examination. Informed consent was obtained from all participants at the beginning of the study, which was performed according to the Declaration of Helsinki. The study was approved by the Local Ethics Committee of Balikesir University Medical School (date: 27.01.2021, approval number: 2021/16).

Study Design and Population

Two hundred and thirty-four participants were allocated into two groups (fatty liver group and healthy control group) according to liver ultrasound (US). The presence of active infection, pregnancy, history of alcohol consumption >140 g/week, abnormal iron and copper metabolism, abnormal thyroid function tests, acute or chronic inflammatory diseases, malignancy, chronic drug use (antidiabetic, antilipidemic and antihypertensive drugs, etc.) were the exclusion criteria.

Anthropometric Measurements

All subjects provided a medical history and underwent a clinical examination. The weight and height of the patients were assessed with a standard measuring scale and body mass index (BMI) values were calculated as body weight / height². Waist circumference (WC) was measured with reference to anterior superior iliac crest and the lowest rib. Blood pressure (BP) was measured three times in the seated position (Omron brand M2 sphygmomanometer), and the mean value was registered.

Definition of Metabolic Disorders

The participants with Hepatosteatosi, established based by US, were defined as MAFLD group in the presence of any one of the following three conditions: presence of Type 2 Diabetes Mellitus (T2DM), overweight/obesity, or evidence of metabolic dysregulation⁴. The presence of metabolic dysregulation was defined as having two or more criteria listed here:

(1) WC \geq 102 cm in male and \geq 88 cm in female; (2) BP \geq 130/85 mmHg or treatment of previously diagnosed hypertension (HT); (3) triglycerides (TG) \geq 1.70 mmol/l or treatment of previously diagnosed dyslipidemia; (4) high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L for male and <1.3 mmol/L for female; (5) prediabetes [fasting plasma glucose (FPG) levels 5.6 to 6.9 mmol/L, or 2-hour post-load glucose levels 7.8 to 11.0 mmol/L or hemoglobin A1c 5.7% to 6.4%]; (6) homeostasis model assessment of IR (HOMA-IR) level \geq 2.5; and (7) C-reactive protein level >2 mg/L.

A subject with three or more criteria for metabolic disorder listed here was defined as MetS according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III)¹⁵: (1) WC \geq 102 cm in males and \geq 88 cm in females; (2) BP \geq 130/85 mmHg or treatment of previously diagnosed HT; (3) TG \geq 1.70 mmol/L

or treatment of previously diagnosed dyslipidemia; (4) HDL-C <1.0 mmol/L for male and <1.3 mmol/L for female; (5) FPG \geq 100 mg/dL or treatment of previously diagnosed T2DM.

Biochemical Analysis

Blood samples were taken from an antecubital vein after 8 hours of fasting. Complete blood count and subgroup analyses were performed using an autoanalyzer [Beckman coulter LH 780 hematology analyzer (Beckman Coulter, Inc., USA)]. FPG, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), TG and HDL-C levels were measured by the enzymatic colorimetric method [Beckman coulter AU 680 chemistry analyzer (Beckman Coulter, Inc., USA)]. Plasma MOTS-c level was measured by the ELISA method (MOTS-c direct ELISA kit, SunRed Biotechnology Company, China). According to the data reported by the manufacturing company, the intra-assay coefficient of variation (CV) ranged from <9%, while inter-assay CV ranged from <11% for the MOTS-c assay.

Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula [$LDL-C = TC - (HDL-C + TG/5)$]¹⁶. The serum basal insulin level was measured by the chemiluminescence method [Immunoassay UniCel DXI800 (Beckman Coulter, Inc., USA)]. Modified HOMA-IR was used to assess insulin resistance [$HOMA-IR = \text{fasting plasma insulin } (\mu\text{U/ml}) \times \text{FPG (mg/dl)}/405$]¹⁷.

Noninvasive Scores of Fibrosis

In this study, NAFLD fibrosis score (NFS) and fibrosis 4 (FIB-4) were used to determine the level of fibrosis². The NFS was calculated using a formula consisting of six variables [$NFS = -1.675 + 0.037 \times \text{age (year)} + 0.094 \times \text{BMI (kg/m}^2) + 1.13 \times \text{impaired fasting glycemia/DM (yes=1; no=0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelets } (\times 10^9/\text{L}) - 0.66 \times \text{albumin (g/dL)}$]. The FIB-4 was evaluated using the formula: $FIB-4 = \text{Age (years)} \times \text{AST (U/L)}/[\text{platelets } (\times 10^9/\text{L}) \times \text{ALT}^{1/2} (\text{U/L})]$.

Liver US

All liver US examinations were performed with an SSA 780A US scanner (Toshiba, Tokyo, Japan) and a 1-6 MHz broadband convex transducer. Liver US studies were performed by the same researcher (BY) with experience in imaging diagnosis of fatty liver. The patients were placed in the supine position during the evaluation. The normal hepatic

parenchyma has a homogeneous and equal echogenicity to the renal cortex. The hepatosteatosis was described as an increase in the liver parenchyma echogenicity according to the renal cortex¹⁸.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Science 15.0 package program (SPSS, Inc., Chicago, IL, USA). Continuous data were expressed as mean \pm standard deviation or median (interquartile range). Categorical variables were expressed as numbers with percentages. Shapiro-Wilk test was used to test normality distribution. Continuous variables were compared between the groups using the *t*-test or Mann-Whitney U test, and categorical variables were compared using the χ^2 test. The relationship between continuous variables was analyzed by Pearson's or Spearman's correlation analysis. Weighted linear regression analysis was performed to assess the independent relationships between the MOTS-c level and the explanatory variables. The *p*-value <0.05 was considered statistically significant.

Results

The demographic, clinical, and laboratory parameters of patients with MAFLD [*n*=150; median age 41.0 (14) years; males 38%] and controls [*n*=84; median age 36.0 (22) years; males 40.4%] are shown in Table I. Patients with MAFLD had higher BMI, WC, systolic BP (SBP), diastolic BP, FPG, uric acid, TG, insulin, HOMA-IR and ALT levels (*p*<0.05 for all) and lower albumin (*p*=0.013) and MOTS-c levels (*p*=0.009) than the controls.

Furthermore, all participants were divided into two groups according to the presence of MetS. The MOTS-c level was significantly lower in subjects with MetS (*n*=48) compared to those without MetS (*n*=186) (*p*=0.01).

In the total population (*n*=234), MOTS-c levels negatively correlated with the presence of MAFLD ($\beta = -0.229$, *p*<0.001), age ($r = -0.296$, *p*<0.001), BMI ($r = -0.221$, *p*=0.001), WC ($r = -0.286$, *p*<0.001), SBP ($r = -0.134$, *p*=0.04), FPG ($r = -0.250$, *p*<0.001), HOMA-IR ($r = -0.147$, *p*=0.025), TG ($r = -0.159$, *p*=0.015), NFS ($r = -0.216$, *p*=0.001) and FIB-4 ($r = -0.196$, *p*=0.003) (Table II).

Weighted linear regression analysis showed age ($t = -2.385$, 95% CI: -16.0 to -1.5, *p*=0.018) and WC ($t = -0.50$, 95% CI: -14.20 to -1.01, *p*=0.024) were the independent predictors of MOTS-c level.

Table 1. Comparison of clinical and biochemical features of patients with MAFLD and healthy controls.

Variables	Total Group (n=234)	Control group (n=84)	MAFLD group (n=150)	p-value
Age (years)	40.0 (17)	36.0 (22)	41.0 (14)	0.070
Gender [male n (%)]	91 (39.3)	34 (40.4)	57 (38)	0.581
BMI (kg/m ²)	28.9 (7.5)	24.2 (5.88)	31.0 (6.05)	<0.001
WC (cm)	97.68±14.02	87.3±13.0	103.5±10.9	<0.001
SBP (mmHg)	119.0 (20)	115.0 (14)	124.0 (20)	<0.001
DBP (mmHg)	76.0 (10)	70.0 (13)	80.0 (10)	<0.001
FPG (mg/dL)	96.0 (13)	93.50 (11)	97.0 (13)	0.006
GFR (mL/min)	93.1±13.5	92.2±16.0	93.7±12.0	0.460
Creatinine (mg/dL)	0.86 (0.20)	0.90 (0.23)	0.86 (0.18)	0.059
Uric acid (mg/dL)	5.0 (1.8)	4.7 (2.0)	5.1 (1.9)	0.002
Albumin (g/dL)	4.58±0.29	4.64±0.25	4.55±0.31	0.013
TC (mg/dL)	198.48±39.02	192.94±36.22	201.59±40.29	0.090
LDL-C (mg/dL)	120.50±34.16	117.52±32.43	122.17±35.09	0.320
HDL-C (mg/dL)	51.0 (14.25)	52.50 (15)	50.0 (16)	0.060
TG (mg/dL)	112.50 (83.5)	93.50 (54)	133.00 (92)	<0.001
Insulin (μU/mL)	8.13 (6.6)	5.98 (3.3)	10.57 (7.0)	<0.001
HOMA-IR	1.96 (1.8)	1.37 (0.79)	2.56 (1.94)	<0.001
ALT (IU/L)	19.50 (14)	16.50 (10)	22.00 (17)	<0.001
AST (IU/L)	18.50 (8)	18.00 (7)	19.00 (10)	0.247
MOTS-c (pg/mL)	217.10 (652.5)	343.95 (1,073.6)	191.93 (384.9)	0.009
NFS	-3.07 (1.68)	-3.24 (1.28)	-3.05 (1.76)	0.385
FIB-4	0.59 (0.40)	0.69 (0.45)	0.57 (0.40)	0.117

The data is given in mean±SD or median (interquartile range). MAFLD: Metabolic dysfunction-associated fatty liver disease, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, GFR: glomerular filtration rate, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, HOMA-IR: homeostatic model assessment-insulin resistance, ALT: alanine aminotransferase, AST: aspartate aminotransferase, MOTS-c: mitochondrial open reading frame of the 12s ribosomal RNA type-c, NFS: non-alcoholic fatty liver disease fibrosis score, FIB-4: Fibrosis-4. Bold values: $p < 0.05$, statistically significant.

Discussion

The results of the present study show that people with MAFLD have lower plasma MOTS-c levels when compared to the healthy control subjects. Lower MOTS-c levels were significantly correlated to the presence of MAFLD and the increased noninvasive scores of fibrosis. Furthermore, circulating MOTS-c levels were lower in subjects with MetS compared to those without MetS and negatively correlated with the components of MetS. Older age and visceral obesity were the significant independent determinants of the MOTS-c levels. These findings imply that the decreased MOTS-c levels may reflect the unhealthy metabolic profile, MetS, MAFLD, and even liver fibrosis.

The classical paradigm about mitochondria which limits its role only to energy metabolism, is significantly changed in recent years⁶. Emerg-

ing data^{6,9} has shown that mitochondria are involved in glucose, amino acid, and lipid metabolism *via* several signaling peptides. MOTS-c is a novel anti-inflammatory and anti-apoptotic mitochondrial peptide that preserves mitochondrial function and cellular metabolism under intrinsic or extrinsic stress conditions¹⁹. *In-vitro* studies^{6,20} show that MOTS-c improves glucose metabolism by increasing glucose clearance and intracellular glucose levels. MOTS-c treatment in mice lowers islet infiltration, improves blood glucose levels, and delays DM progression in non-obese diabetic mice²¹. MOTS-c inhibits the expression of the pro-inflammatory cytokines and improves endothelial function in the aortic explants of animal models^{22,23}. Administration of MOTS-c leads to increased brown fat activation in white adipose tissue, lower fatty acid levels in serum and liver, and improves insulin resistance²⁴.

Table II. The analysis of the correlation between circulating MOTS-c levels and continuous parameters.

Variables	r	p-value
Age	-0.296	<0.001
BMI	-0.221	0.001
WC	-0.286	<0.001
SBP	-0.134	0.04
DBP	-0.100	0.128
FPG	-0.250	<0.001
Insulin	-0.115	0.08
HOMA-IR	-0.147	0.025
GFR	0.201	0.002
Uric acid	-0.72	0.275
ALT	-0.08	0.216
AST	-0.055	0.416
TC	-0.101	0.110
LDL-C	-0.109	0.097
HDL-C	0.100	0.129
TG	-0.159	0.015
NFS	-0.216	0.001
FIB-4	-0.196	0.003

MOTS-c: mitochondrial open reading frame of the 12s ribosomal RNA type-c, BMI: body mass index, WC: waist circumference, SBP: systolic blood pressure, DBP: diastolic blood pressure, FPG: fasting plasma glucose, HOMA-IR: homeostatic model assessment-insulin resistance, GFR: glomerular filtration rate, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, NFS: non-alcoholic fatty liver disease fibrosis score, FIB-4: Fibrosis-4. Bold values: $p < 0.05$, statistically significant.

MAFLD is the most common chronic liver disease, which is defined as a condition characterized by liver fat accumulation in the presence of overweight/obesity or T2DM, or two or more metabolic risk factors⁴. The increased liver fat accumulation not only progresses to steatohepatitis and fibrosis over the years but also constitutes a significant risk for cardiovascular mortality^{2,25}. The new definition of MAFLD constitutes a broader definition and represents a greater cardiovascular diseases risk burden than the previous terminology of NAFLD²⁶. Due to the high prevalence of MAFLD, there is a need for reliable non-invasive methods for predicting fibrosis in these patients. Animal studies show that MOTS-c treatment reduces plasma free fatty acids and liver fat accumulation^{6,12,24}. However, no human study has been published to search for the relationship of MOTS-c to the presence of MAFLD and its severity. To the best of our knowledge, the present study is the first to show lower circulating MOTS-c levels in patients with MAFLD. In addition, lower MOTS-c

levels were significantly correlated to the fibrosis scores (NFS and FIB-4) in subjects with MAFLD. Overall, MOTS-c levels were negatively correlated to all the parameters of unhealthy metabolic profiles such as older age, higher BMI, WC, SBP, FPG, TG, and HOMA-IR levels.

The negative association of MOTS-c with MetS and related diseases was not previously unanimously shown. Low plasma MOTS-c levels were reported in obese male children and adolescents, while high concentrations of MOTS-c were reported in the blood of mothers and newborns^{27,28}. Another study²⁹ reported low MOTS-c levels in patients with T2DM and even lower levels in those with poorer metabolic control, while a positive association was reported between the MOTS-c levels and the BMI in the same study. However, controversial to these reports, another study³⁰ mentioned that the components of MetS were significantly positively correlated to the MOTS-c levels. We think that the inconsistencies in these studies were likely due to differences in enrolled patient populations, particularly by age and number of participants. Our data seem to be consistent, showing that there is a decrease in MOTS-c levels in parallel to the impaired metabolic profiles of an adult population. Our results showing the role of age and visceral obesity as the significant independent determinants of low MOTS-c levels account for the previous reports²⁰ about the role of MOTS-c in human aging and age-related diseases.

Limitations

This study has several limitations. Firstly, the study was a single-center experience, and the number of subjects was not large enough to make detailed inferences. Secondly, due to the cross-sectional design of the study, it was not possible to make assumptions about the causality of the relationship between low MOTS-c levels and MAFLD. Lastly, HOMA-IR method may not be the gold standard method for the measurement of insulin sensitivity and nor does US for the diagnosis of fatty liver.

Conclusions

In conclusion, our study shows that circulating MOTS-c levels are lower in subjects with MetS, MAFLD and negatively correlated to the noninvasive scores of liver fibrosis. MOTS-c levels are significantly and consistently associated with the components of MetS. This pilot study is the first to investigate the possible role of MOTS-c in the

pathogenesis of MAFLD and other metabolic diseases in humans. Further studies with larger case numbers are expected to better define the role of MOTS-c in the pathogenesis of MAFLD and chronic metabolic diseases.

Conflict of Interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Ethics Approval

This study was conducted in accordance with the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of Balikesir University Medical School on 27.01.2021 with 2021/16 decision number.

Authors' Contributions

A.S., T.D., and A.K.; methodology: A.S., T.D., and A.K.; Data curation: O.B., B.Y., H.S., M.E., E.A.B, A.E., E.S., and A.K; investigation: A.S., T.D., and A.K.; formal analysis: I.T.; writing-original draft: A.S., T.D. and A.K; writing-review and editing: A.S., T.D., and A.K. All authors have read and agreed to the published version of the manuscript.

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