Comparison of Serum MOTS-c Levels in Patients with Multiple Sclerosis and Healthy Controls

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Abstract

Background

Multiple sclerosis (MS) is a major global problem as its pathogenesis is understood more clearly, therapeutic options expand accordingly. Mitochondrial open-reading-frame of the twelve S rRNA–c (MOTS-c) is a novel mitochondria-derived protein acting on metabolic homeostasis.

Objective

The main objectives of this report are giving an idea of the role of serum MOTS-c in the pathophysiology of the disease in MS patients, investigating whether the mechanism of MOTS-c.

Methods

Forty-three patients diagnosed with relapsing-remitting MS and 41 healthy controls were enrolled in the study. MOTS-c, fasting blood glucose, insulin, HOMA-IR, lipid panel, and body-mass index were assessed.

Results

The patients' MOTS-c levels remained significantly lower than the controls, while their fasting blood glucose and HOMA-IR values were higher. Our multivariate logistic regression analysis established that increased MOTS-c levels could be a protective factor from the development of MS disease. The area under the ROC curve for MOTS-c was calculated as 0.782 (95% CI: 0.684–0.879, $p = 0.0001$).

Conclusions

Our study is the first to scrutinize MOTS-c levels in MS patients. We tried to provide clinical evidence that MOTS-c could act as a highly discriminative biomarker between MS patients and controls and hold great promise for new therapeutic options.

Introduction

Multiple sclerosis (MS), an immune-mediated, demyelinating, and neurodegenerative disease, is more prevalent among women, often among young adults. While the pathogenesis of MS is still full of mysteries, low vitamin D levels, Epstein-Barr virus infection, smoking, exposure to ultraviolet-B light, and obesity constitute risk factors responsible for its pathogenesis [1]. In addition to these environmental factors, genetic background is also a major determinant of MS development.

It is well-documented that diabetes mellitus (DM), cardiovascular diseases, and vascular disease risk factors, such as hyperlipidemia and hypertension (HT), which are more prevalent in MS patients, adversely affect the clinical progression of MS[2,3]. Published clinical trials report that childhood and adolescent obesity might heighten the risk of MS development and progression [4,5].

Autoreactive T-lymphocytes, B-lymphocytes, macrophages, activation of microglial cells, various antibodies, activated complements, cytokines, mitochondrial dysfunction, presence of metalloproteinases, and their induced oxidative stress are all among the underlying mechanisms responsible for neuronal and axonal damage of the brain and spinal cord in MS [6].

Insulin resistance (IR) is a chronic inflammatory process aggravating neuroinflammation linked to diseases, such as MS [7]. IR and oxidative stress are closely interrelated within a vicious cycle. Multiple lines of evidence suggest that increased secretion of pro-inflammatory cytokines induced by oxidative stress can promote IR, which also induces oxidative stress [8]. IR and oxidative stress are reported to be more prevalent among MS patients than controls and to exacerbate the progression in these patients [9].

MOTS-c is a novel mitochondria-encoded peptide, circulating and involving in some endocrine activities. MOTS-c, found in tissues such as muscle, brain, and liver, can be measured from plasma, cerebrospinal fluids [10]. Thus far the reported results have revealed that MOTS-c levels are associated with IR and obesity markers, including body-mass index (BMI), waist circumference, waist-to-hip ratio, fasting insulin level, HOMA-IR, and HbA1c [11].

MOTS-c has the potential to yield numerous metabolic benefits. The clinical experiments performed so far have confirmed that these effects have affinity with AMP-activated protein kinase (AMPK) activity, elevate endogenous 5-Aminoimidazole-4-carboxamide-1-β-d-riyafuranoside (AICAR) levels, and inhibit oxidative phosphorylation while enhancing glucose utilization and fatty acid oxidation. MOTS-c may also accelerate muscular glucose uptake in response to insulin production and modulate metabolites to regulate insulin sensitivity in mice fed a high-fat diet, thereby promoting insulin sensitivity [10].

As identified by recent clinical research, there is a clear association between MOTS-c and obstructive sleep apnea syndrome, polycystic ovary syndrome, and vascular risk factors, such as endothelial dysfunction [12,13,14]. In this context, the aim of our research was to further current knowledge of MOTS-c levels in MS disease, whose pathogenesis has not been fully elucidated yet and which is associated with many metabolic abnormalities. Thus, we hoped to contribute to the literature through our findings and shed new light on both pathogenesis and treatment of MS.

Methods

Study design and participants

Forty-one healthy volunteers and 43 patients diagnosed with relapsing-remitting MS aged 18–75 years who presented to the neurology department of a tertiary-level hospital in XXX, were enrolled in our study. Neurological examinations of all the patients were performed prospectively, and their demographic data as well as expanded disease severity scale (EDSS) scores were recorded. Exclusion criteria involved presence of other accompanying neurodegenerative disorders, chronic alcohol and drug abuse, and progressive MS diagnosis. Informed consent forms were obtained from all the enrolled participants in our study conducted in accordance with the guidelines recommended by Medical Ethics Committee of Pamukkale University with dated 02.03.2021 and numbered 05.

Procedure

Initially, approximately 5 ml of venous blood was drawn into biochemistry tubes containing SST gel from the patients and the healthy controls following at least an 8-hour fast in the morning. The serum specimens obtained after centrifugation at 3500 rpm for 10 min were transferred into Eppendorf tubes and stored at -80 degrees until the trial day. MOTS-c levels were analyzed from the prepared serum at room temperature on the trial day in the research laboratory of the Department of Medical Biochemistry by using a commercial competitive ELISA kit (IT Laboratory-Shanghai, China).

After the standards and chemicals of the kits used in the study were prepared, the standards and serum samples were pipetted into the wells in the microplate. By following the steps described in the instructions, the serum samples were colored based on the concentrations of the tests. After color formation was observed, the absorbance values of the wells were read at 450 nm using a Biotek Elx800 Microplate reader (BioTek Instruments Inc., USA). The concentrations were calculated using the serum absorbance values on the Gen5 data analysis program. The resulting values were expressed as ng/ml.

Statistical Analysis

All the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) v.25 (IBM Corp., Armonk, NY, USA). The continuous variables were reported as mean ± standard deviation and the categorical variables as number and percent. Normality of the data was checked by a Shapiro-Wilk test. The independent group comparisons were calculated using a Mann-Whitney U test, and a Spearman correlation analysis was performed to investigate the relationships between continuous variables. Univariate and Multiple Logistic Regression Analysis was used to identify which variables triggered the development of MS disease. The difference between the categorical variables was analyzed by chi-square test. ROC analysis was conducted to identify presence of MS and optimal cut-off value for MOTS-c levels. Statistical significance was set at $p < 0.05$.

Results

Forty-three patients diagnosed with relapsing-remitting MS and 41 healthy volunteers with no history of neurological disorders were recruited in our study. The mean age of the patients and the controls was

37.47±10.41 and 38.05±10.87, respectively. No significant difference was noted between the patient and the control group in terms of age and gender (p> 0.05).

The patient population included 33 females (76.7%) and 10 males (23.3%), while the control group consisted of 31 females (75.6%) and 10 males (24.4%). The mean BMI scores of the patients and the controls corresponded to 25.32±5.41 and 25.88±4.54, respectively, which did not reveal a significant difference (p> 0.05).

As far as comorbid conditions are concerned, 8 patients (18.6%) were diagnosed with DM, 6 (14.0%) with hyperlipidemia, and 3 (7%) with hypertension, yet only the presence of DM was significantly higher in the patients than the controls (p = 0.03). In addition, the mean EDSS score and number of attacks of the patient group were 2.21±1.28 and 3.84±2.24, respectively. Thirteen patients (30.2%) were receiving immunomodulator therapy, 12 (27.9%) glatiramer acetate therapy, 8 (19.6%) oral first-line treatment, and 10 (23.3%) fingolimod treatment. **Table 1** summarizes the clinical and demographic information of the patients.

When the laboratory data of both cohorts were compared, MOTS-C (p=0.0001), fasting blood glucose (p = 0.034), and HOMA-IR values (p = 0.027) were observed to differ significantly. The mean MOTS-c level of the patients was 159.23 ± 40.79, which was significantly lower than that of the controls (189.95 ± 22.51) (p = 0.0001). The mean values for fasting blood glucose and and HOMA-IR in the patients was identified as 105.6 ± 30.56 and 3.58 ± 2.82, respectively, both of which were significantly higher than the controls (p = 0.034, p = 0.027). On the other hand, no significant difference was evident between the patients and controls regarding blood lipids, folic acid and vitamin B12 levels (p> 0.05). **Table 2** provides an overview of the laboratory data belonging to the patients. However, a positive correlation was obtained between MOTS-C values and those of non-HDL (r = 0.423, p = 0.006), VLDL (r = 0.048, p = 0.003), triglyceride (r = 0.489, p = 0.001) and total cholesterol (r = 0.369) (p = 0.018) in the control group **(Table 3)**. In addition, no correlation was observed between MOTS-C and folic acid and vitamin B12 levels in both cohorts (p> 0.05).

No significant difference was found between males and females in relation to MOTS-c values of both groups (p> 0.05).

A multiple logistic regression model was developed based on factors with a significant effect on MS presence. As is evident from this model, the increase in MOTS-c was a protective factor from MS disease, independent of DM presence and HOMA-IR effect (P = 0.002; O.R: 0.957) **(Table 4)**.

Regarding the discrimination of MOTS-c values for MS, the area under the ROC curve (AUC) was found as 0.782 (95% CI: 0.684-0.879, p = 0.0001). Accordingly, if the optimum cut-off point defined according to Youden Index values was set as 190.5, the presence of MS disease could be predicted at 88.4% sensitivity and 56.1% specificity **(Figure 1)**.

Discussion

The most important clinically relevant finding in our study is significantly lower MOTS-c values in MS patients than in the controls. DM, obesity, hyperlipidemia, and vascular comorbidities, such as coronary artery disease, are more prevalent among MS patients. These comorbid conditions are assumed to act on MS pathogenesis and may further exacerbate disability when accompanying MS [2,3]. The interaction between MOTS-c values and some disorders is well-documented, with previous research indicating that MOTS-c values remained low in patients with obesity, insulin resistance, DM, and endothelial dysfunction [14,15,16]. Consistent with the literature, our results also reveal that MOTS-c values were lower in MS patients than in healthy controls. The lack of a significant difference between both cohorts regarding age, gender and BMI shows that MOTS-c could be a discriminating indicator in MS, independent of the above-mentioned variables.

MOTS-c is a 16-amino-acid peptide synthesized from the mitochondrial 12S rRNA region [10]. Mitochondria constitute a fundamental system that maintains metabolic functions by regulating cellular energy homeostasis. However, mitochondrial dysfunction triggers deficiency in the electron transport chain, beta-oxidation, and associated IR [16]. A clinical trial on rodents in relation to the metabolic regulation effects of MOTS-c suggests that the activation of a mechanism linked to AMPK enhances glucose utilization in skeletal muscle, improving insulin sensitivity and accelerating restoration of metabolic homeostasis [17]. The chronic inflammatory process in MS induces mitochondrial DNA mutations through the production of reactive oxygen species, eventually resulting in mitochondrial damage. This metabolic stress triggers protein misfolding in the endoplasmic reticulum, disruptions in energy production, neuronal losses, and ultimately neuroaxonal dysfunction [6]. All these processes in close connection to mitochondrial dysfunction can account for lower MOTS-c levels in MS that we revealed in our study.

The findings we obtained from our clinical trials established that fasting blood glucose and HOMA-IR values as well as the presence of DM were significantly higher in MS patients than the healthy controls. MS heightens the risk of developing Type 1 DM and increases the incidence of Type 2 DM in comparison to the general population [7]. This is assumed to emerge as a result of the IR triggered by the cytokines which arise due to MS-induced autoimmune processes and chronic inflammation. Previous reports have also documented that IR is likely to drive disability worsening in MS patients [9]. In addition, our univariate analysis revealed that DM, fasting blood glucose, insulin, HOMA-IR, and MOTS-c became significant contributors to MS development. Insulin abnormalities may also enhance inflammatory response and oxidative stress [18]. Peripheral hyperinsulinemia leads to increased insulin secretion in the cerebrospinal fluid. Paradoxically, persistent hyperinsulinemia downregulates insulin receptors at the blood-brain barrier, reducing insulin delivery to the brain. Thus, IR-associated peripheral hyperinsulinemia brings about hypoinsulinemia in the central nervous system, which impairs both cognition and selective brain function [7]. In relation to the protective effect of insulin on the brain, neurodegenerative diseases, such as MS, may arise as a result of decreased central insulin.

Higher HOMA-IR values in our patient group with low MOTS-c values in comparison to the controls confirm the results reported by previous studies highlighting the interaction between MOTS-c and IR [11,12].

A substantial body of experimental and clinical research lends support to the interplay between plasma MOTS-c concentration and increased insulin sensitivity [10,15,19]. Overexpression of MOTS-c enhances glucose uptake in myoblasts. A study conducted on mice concluded that intraperitoneal intervention of MOTS-c could regulate age and diet-dependent IR by activating the AMPK pathway in skeletal muscle and enhancing GLUT4 expression [17]. The impact of MOTS-c on increasing insulin sensitivity is well-established, but as our multivariate analysis reveals, increased MOTS-c level may exert a protective effect on MS development once DM and HOMA-IR factors are excluded. This finding suggests that MOTS-c is involved in the pathogenesis of MS in a different fashion.

Our results did not yield a significant difference between both cohorts in terms of BMI values. Previous research likewise did not report a marked difference between MS patients and healthy individuals regarding BMI but found higher BMI values in MS patients with IR than in individuals without IR [9]. They attributed this to the fact that oxidative stress in the pathogenesis of MS is not triggered by overweight alone but also by other underlying mechanisms and may contribute to weight gain.

While we did not observe a strong relationship between MOTS-c values and blood lipids in our patient cohort, a significant positive correlation was noted between MOTS-c and non-HDL, VLDL, TG, and total cholesterol values in the controls. Published research also reported a positive correlation between circulating MOTS-c and LDL and total cholesterol levels [17]. In addition, it has been noted that increased lipid production enhances MOTS-c expression while insulin inhibits lipid-induced MOTS-c [13]. Lower IR in our control cohort was not a significant contributor to the relationship between MOTS-c and lipids. By contrast, the higher IR in our patients tended to suppress the lipid-induced MOTS-c expression, blocking the interaction between MOTS-c and lipids. In fact, this critical difference is suggestive of the involvement of different processes affecting working mechanism of MOTS-c in MS patients.

While some studies suggest that MOTS-c interacts with folate-methionine cycle and inhibits folate production [10], others report that MOTS-c does not target the folate cycle [19]. Similarly, we did not identify a significant interplay between MOTS-c and folate levels in our study.

Healthy skeletal muscles have a metabolic flexibility that can change glucose and fat utilization on an as-needed basis. Dodecanedioic acid (DAC), a dicarboxylic acid, acts as an alternative energy source when metabolic flexibility is lost [20]. MOTS-c has been reported to boost energy capacity [21], reduce muscular fatigue, and improve physical performance by increasing DAC uptake in skeletal muscle during exercise in obese mice [19]. Fatigue is one of the prevalent clinical manifestations of MS that profoundly degrades the quality of life. It should be noted that low MOTS-c levels and fatigue might be interrelated at the molecular level, and MOTS-c treatment can potentially eliminate this symptom. The insights provided so far into MS pathogenesis and course have helped to make a tremendous advance in MS treatment. However, current treatments provide only partial protection against neurodegenerative components of MS. Further research on the merit of early treatment and powerful agents will be important for evidence-

based approaches to treating and managing MS [22]. Clinical trials on the utilization of MOTS-c as a treatment tool have yielded very promising results thus far [23].

Similar to prior studies [11,17], our study did not detect any significant difference between genders in MOTS-c levels. Although some research noted a difference in the obese male children relative to the controls, no difference existed between genders [11].

In our ROC analysis, we found that when the area under the ROC curve of MOTS-c was 0.782, its sensitivity was quite high, though specificity was low at this value. This indicates that MOTS-c might act as an effective biomarker in diagnosing MS but falls short of distinguishing the healthy group.

Limitations

A major limitation of this study lies in the fact that our patient cohort consisted of only RRMS patients, leading us not to evaluate the course of MOTS-c in progressive patients. Secondly, the fact that EDSS was not at a very advanced stage restricted our evaluation of the impact of MOTS-c on advanced disability. MOTS-c levels should be addressed in a larger and clinically diverse patient population in future studies.

Conclusions

We tried to provide clinical evidence that MOTS-c could be a highly discriminative biomarker between MS patients and controls, which might hold great promise for new therapeutic options. In addition, the finding that overexpression of MOTS-c can be a protective factor from MS development after excluding DM and HOMA-IR effect is suggestive of its involvement in MS pathogenesis through different mechanisms. In this sense, there is need for further studies to be planned experimentally in the future in order to confirm that, with its effect on MS pathogenesis, MOTS-c can be protective against fatigue, attack and progression.

Declarations

Author Contributions

S.T. Conceptualization, Methodology, Investigation, Writing – original draft. **E.A.** Investigation, Writing – review & editing. **I.T.** Investigation, Writing – review & editing. **H.S.** Statistical Analysis. **U.C**. Resources**.** **L.S.B.** Supervision.

Competing Interests

There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Additional Informations

Correspondence and requests for materials should be addressed to **S.T**

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Tables

Table 1. Demographic and clinical findings of subjects

		MS (n=43)	Control (n=41)	p value
Age		38.05 ± 10.87	37.47 ± 10.41	0.971
Gender	male / female	10 (23.3%) / 33 (76.7%)	10 (24.4%) / 31 (75.6%)	0.903
BMI		25.32 ± 5.41	25.88 ± 4.54	0.388
Diabetes Mellitus	- / +	35 (81.4%) / 8 (18.6%)	40 (97.6%) / 1 (2.4%)	**0.003***
Hypertension	- / +	40 (93%) / 3 (7%)	38 (92.7%) / 3 (7.3%)	1
Hyperlipidemia	- / +	37 (86%) / 6 (14%)	35 (85.4%) / 6 (14.6%)	0.929
Cardiac disease	- / +	43 (100%) / 0 (0%)	41 (100%) / 0 (0%)	-
EDSS		2.21 ± 1.28		
Treatment types	Immunmodulator	13 (30.2%)		
	Glatiramer Acetate	12 (27.9%)		
	Dymethil-fumarate	4 (9.3%)		
	Teriflunomide	4 (9.3%)		
	Fingolimode	10 (23.3%)		

Abbreviations: MS,Multiple Sclerosis; BMI, body mass index; EDSS, expanded disease severity scale

*** p< 0.05**

Table 2. The laboratory findings of MS patients and control groups

	MS (n=43)	Control (n=41)	p value
	Mean ± S.D.	Mean ± S.D.	
Glucose (mg/dl)	105.6 ± 30.56	92.34 ± 8.82	**0.034***
Insülin (mU/L)	13.46 ± 10.06	9.03 ± 3.98	0.093
HOMA-IR	3.58 ± 2.82	2.08 ± 1.02	**0.027***
Non-HDL Cholesterol (mmol/L)	138.28 ± 39.12	128.44 ± 32.79	0.594
LDL Cholesterol (mmol/L)	109.3 ± 30.26	102.59 ± 25.9	0.754
HDL Cholesterol (mmol/L)	50.26 ± 11.69	53.29 ± 10.48	0.202
Total Cholesterol (mmol/L)	187.28 ± 36.66	179.2 ± 31.93	0.71
Triglycerides (mmol/L)	138.14 ± 64.68	123.71 ± 69.46	0.263
VLDL Cholesterol (mmol/L)	27.72 ± 13	25.41 ± 13.76	0.383
Folic_Acid (ug/L)	8.33 ± 3.39	8.73 ± 3.6	0.658
Vitamine B12 (ng/L)	393.76 ± 208.47	358.64 ± 103.86	0.904
MOTS-c	159.23 ± 40.79	189.95 ± 22.51	**0.0001***

Abbreviations: MS,Multiple Sclerosis; HOMA-IR, Homeostatic model assessment indicator of insulin resistance; ; HDL, high-density cholesterol; LDL, low-density cholesterol; VLDL, very low-density cholesterol; MOTS-c, Mitochondrial open-reading-frame of the twelve S rRNA–C.

*** p< 0.05**

Table 3. The significant correlation between MOTS-c and lipid profile in control group

	MOTS-c
Non-HDL	p = 0.006*; r = 0.423
VLDL	p = 0.003*; r = 0.448
TG	p = 0.001*; r = 0.489
Total Cholesterol	p = 0.018*; r = 0.369

Abbreviations: non-HDL, non-high-density cholesterol; VLDL, very low-density cholesterol; TG, Triglycerides

*** p< 0.05**

Table 4. Univariate and Multiple Logistic Regression Analysis Results

		Wald	p	O.R.	95% C.I.for O.R.	
					Lower	Upper
Multiple	DM	1.085	0.298	0.256	0.02	3.326
	HOMA	3.482	0.062	1.362	0.985	1.884
	MOTS-c	9.969	**0.002***	0.957	0.932	0.984

* p <0.05 statistically significant; O.R: Odds Ratio; C.I: Confidence Interval

Figures

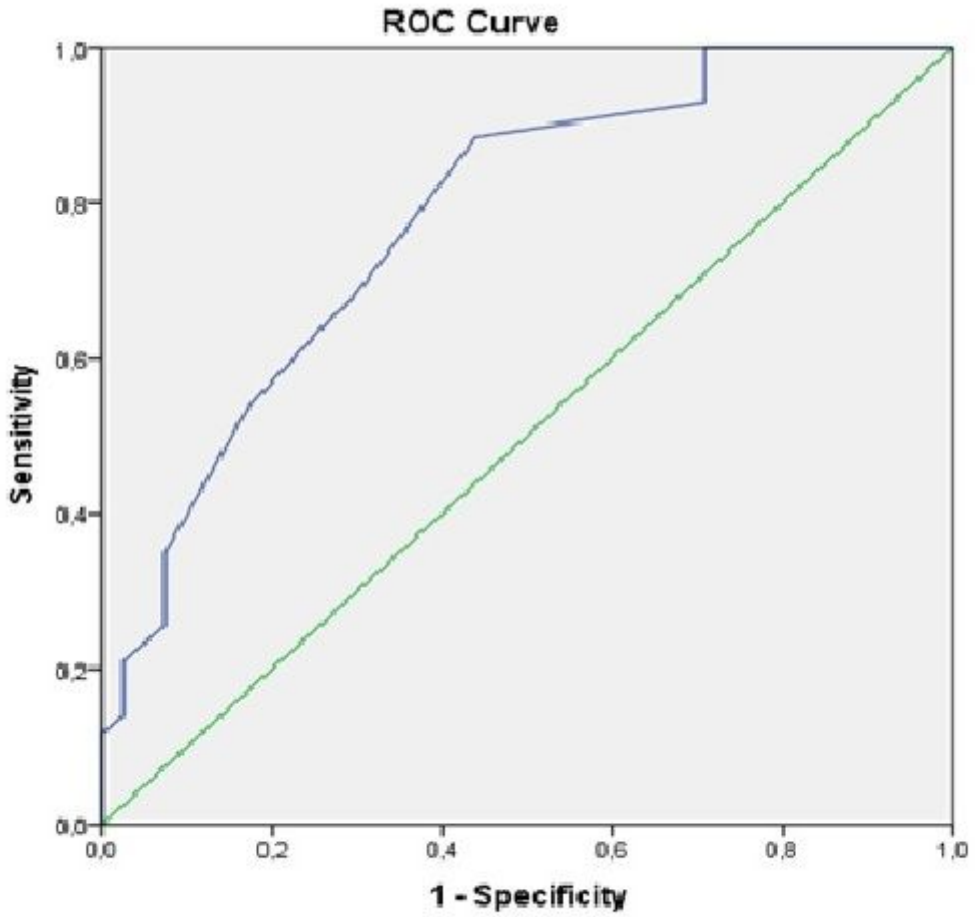

Figure 1

ROC Analysis for MOTS-C results