Research Article

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Gestational diabetes mellitus is associated with a low serum level of mitochondrial-derived peptide-MOTS-C

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Abstract

Objectives: Although MOTS-C has been reported to have a role in diabetes mellitus, no human studies have evaluated the serum level of MOTS-C in GDM. It was aimed to investigate serum levels of MOTS-C in patients with gestational diabetes mellitus (GDM).

Methods: Comparisons were made of 44 pregnant patients diagnosed with GDM and a control group of 44 healthy pregnant women in respect of serum MOTS-C, insulin, and glucose levels, and serum lipid profile.

Results: A significantly higher level of fasting serum glucose and significantly lower serum levels of MOTS-C and high density lipoprotein were determined in the GDM group compared to the control group (p<0.05 for all). A cut-off value of 173.5 ng/mL for serum MOTS-C level had sensitivity of 81.8 % and specificity of 61.4 % for GDM diagnosis (p<0.001). A significant correlation was determined between the serum MOTS-C and serum glucose levels (r=−0.239, p=0.025).

Conclusions: For the first time in literature, the results of this study showed that patients with GDM had a decreased serum level of MOTS-C and that increasing serum MOTS-C levels were associated with a decrease in serum glucose levels, thereby supporting the view that mitochondrial dysfunction plays a role in GDM pathogenesis. Therefore, MOTS-C could be a promising diagnostic biomarker for GDM cases.

Keywords: gestational diabetes mellitus; biomarker; MOTS-C; pregnancy; diagnosis

Introduction

Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed during pregnancy [1]. Although prevalence has been reported as 7–16.5 %, it varies from community to community according to the diagnostic test and diagnostic criteria used [2, 3]. Screening for GDM in pregnancy and diagnosis are important since GDM leads to an increased risk of problems such as pre-eclampsia, a requirement for operative delivery, postpartum haemorrhage for the mother, and stillbirth, macrosomia, polyhydramnios, and neonatal metabolic problems because of birth trauma in respect of the fetus [4, 5]. It has also been shown that for women with GDM there is an increased risk in the long term after pregnancy of the development of diabetes mellitus (DM), cardiovascular diseases and metabolic dysfunction, and that the infants born to these mothers are prone to developing metabolic problems such as obesity and DM [2, 3, 6].

Although the pathophysiological mechanisms causing the development of GDM are not fully known, suggested mechanisms include insulin resistance, oxidative stress and systemic inflammation similar to type 2 DM [7]. It has also been suggested that GDM occurs through not being able to meet the increased need for insulin in pregnancy associated with beta cell dysfunction present before pregnancy [2, 8].

It has also been shown that a reduction in the number and functions of mitochondria in the skeletal muscles plays a role in the etiopathogenesis of GDM and type 2 DM [2, 9, 10].

In electron microscope studies conducted for the first time in 1963, mitochondrial DNA was detected and was defined as circular DNA similar to bacteria. Regions of short open reading frames (sORFs) in mitochondrial DNA were later defined. Several peptides were identified that were encoded by sORFs, which have been shown to have important biological
or >30 kg/m². Patients were excluded from the study if aged <18 years or >40 years, if group regarding age, body mass index (BMI) and duration of pregnancy. Tolerance test during the study period and were similar to the study pregnant women who were screened negative in the 50 g oral glucose control group (Group 2) was formed of 44 randomly selected healthy GDM was made if 2 or more diagnostic criteria were determined [fasting period. The samples were centrifuged at 1500 g for 10 min and kept at −80 °C until assay.

For the following parameters measured in the current study, manufacturer's values were used for the coefficient of variation and detection range values. Analysis of the human serum MOTS-C concentration was performed using the enzyme-linked immunosorbent assay (ELISA) with commercial kits (Shanghai Coon Koon Biotec Co., Ltd, China) according to the manufacturer's instructions. The intra-assay variation coefficient was <10 %, and the inter-assay variation coefficient was <15 %. Test sensitivity was 1.00 ng/mL, with an upper limit of the standard determined as 200 ng/mL. The samples were studied by diluting and the results obtained were multiplied by the dilution coefficient. Therefore, some of the MOTS-C results of the samples were detected to be higher than the upper limit of the test sensitivity. All the measurements were taken on an automated ELISA reader (Thermo Scientific, FINLAND) and analyzed on computer software (SkanIt for Multiscan FC 2.5.1). The absorbance of each well was determined at 450 nm. The average absorbance (Y) values of the standards against the known concentration (X) of the standards were used to plot the standard curve. The results were stated as MOTS-C concentration (ng/mL). Within 30 min of drawing the blood sample into the grey top tube, the serum glucose level was measured photometrically on a Roche Cobas c702 device using the enzymatic reference method with hexokinase and expressed in mg/dL. The detection range was determined as 2–750 mg/dL (0.11–41.6 mmol/L) with intra- and inter-assay coefficient of variation values of 0.9 and 1.8 %, respectively. Serum insulin level was measured using the Electrochemiluminescence method (ECLIKA) on a Roche Cobas e602 device and expressed as IU/L. The measurement range was 2–300 μU/mL, and the coefficient of variation value was 1.9 %. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, (low-density lipoprotein) LDL cholesterol and triglyceride levels were measured with the photometric method on a Roche Cobas c702 biochemical autoanalyzer (Roche Healthcare Diagnostics, Mannheim, Germany) using the commercial kit of the manufacturer. The measurement range for serum total cholesterol level was stated as 3.86–800 mg/dL with a coefficient of variation value of 0.9 %. For the serum HDL cholesterol level, the measurement range was 15–100 mg/dL with a coefficient of variation value of 0.5 %. The measurement range for the serum LDL cholesterol level was 3.87–549 mg/dL with a coefficient of variation value of 0.8 %. For the serum triglyceride level, the measurement range was 45–650 mg/dL, with a coefficient of variation value of 0.9 %.

Materials and methods

This cross-sectional case-control study was conducted in a tertiary-level university hospital between May 2019 and May 2020. Approval for the study was granted by the Institutional Ethics Committee (# 08/2019), and all procedures were applied in accordance with the principles of the Helsinki Declaration. All the study participants provided informed consent.

The study group (Group 1) comprised 44 cases at 24–32 gestational weeks diagnosed with GDM with 2-step GDM screening during the study period. Blood samples were taken from the study group before any medical treatment or diet had been started.

In our antenatal clinic, GDM screening is applied at 24–32 weeks. Patients with a 1 h glucose value of ≥140 mg/dL in the 50 g oral glucose tolerance test (OGTT) were instructed not to make any lifestyle changes and to follow a regular diet for 3 days before the 100 g 3-h OGTT. Women with a positive 100 g OGTT test according to the criteria defined by Carpenter and Coustan were evaluated as having GDM. The diagnosis of GDM was made if 2 or more diagnostic criteria were determined [fasting plasma glucose ≥95 mg/dL (5.3 mmol/L), 1st h ≥180 mg/dL (10 mmol/L), 2nd h ≥155 mg/dL (8.6 mmol/L), 3rd h ≥140 mg/dL (8.6 mmol/L)] [1, 19]. The control group (Group 2) was formed of 44 randomly selected healthy pregnant women who were screened negative in the 50 g oral glucose tolerance test during the study period and were similar to the study group regarding age, body mass index (BMI) and duration of pregnancy. Patients were excluded from the study if aged <18 years or >40 years, if they had a multiple pregnancy, had any systemic, endocrine or inflammatory disease, if they smoked or drank alcohol, or if BMI was <18.5 or >30 kg/m².

Blood samples were taken in the morning following an overnight fasting period. The samples were centrifuged at 1500 g for 10 min and kept at −80 °C until assay.

Statistical analysis

Statistical Package for the Social Sciences version 20.0 software (SPSS IBM Inc., Armonk, NY, USA) was used for statistical analysis. Conformity of the data to normal distribution was analyzed with the Shapiro-Wilk test. In the comparisons of 2 groups, either the Student’s t-test or Mann Whitney U-test was used according to the results of normality tests. In the correlation analysis, either the Pearson test or Spearman was used depending on the results of normality tests. The continuous variables were stated as mean ± standard deviation (SD) values, and categorical variables as number (n) and percentage (%). To find the cut-off level of MOTS-C able to detect GDM with high sensitivity and specificity, receiver operating characteristic (ROC) curve analysis was applied. A value of p<0.05 was accepted as statistically significant.
Results

Both groups were similar in respect of mean age, BMI, gestational age, gravida, and parity (p>0.05 for all) (Table 1). The mean serum fasting glucose levels were determined to be statistically significantly higher in the GDM group than in the control group (p<0.001) (Table 2). The mean serum HDL cholesterol level in the study group was significantly lower than that of the control group (p=0.018). Groups were similar regarding serum fasting insulin level, total cholesterol, LDL cholesterol and triglyceride levels (p<0.05 for all). The serum level of MOTS-C was significantly lower in the study group compared to the control group (153.9 ± 21.0 vs. 180.5 ± 57.0 ng/mL, p=0.005) (Table 2).

When a cut-off value of 173.5 ng/mL was taken, the serum MOTS-C level was found to determine GDM with sensitivity of 81.8 % and specificity of 61.4 % (p<0.001, Area Under Curve: 0.762, 95 % confidence interval (CI): 0.657–0.867) (Figure 1).

A significant positive correlation was determined between the serum MOTS-C and serum glucose levels (r=−0.239, p=0.025). No significant correlation was determined between the serum MOTS-C level and demographic parameters, serum insulin level or serum levels of lipids.

Discussion

This study showed that the serum MOTS-C level was significantly lower in GDM cases than in healthy pregnant women. The serum MOTS-C level was investigated in GDM cases for the first time in the literature. In a study by Catoldo et al., the serum MOTS-C levels were compared in obese and non-obese patients, and the difference in the mean serum MOTS-C level between the 2 groups was determined as 23.6 %. Considering this difference, power analysis was applied to determine the number of cases required in each group for the study. It was determined necessary to have 20 patients in each group for a error of 0.05 and power of the test of 0.95 [20].

MOTS-C (mitochondrial ORF of the twelve S c) is a peptide encoded by mitochondrial DNA. The target organs of MOTS-C are striated muscle and fat tissue. In a mouse model study, 8 h fasting was shown to reduce the MOTS-C level in muscle and plasma. Using the hyperglycemic euglycemic clamp technique, which is the gold standard in the determination of insulin activity, it has been shown that MOTS-C does not decrease glucose synthesis in the liver, but controls glucose metabolism by increasing glucose uptake of striated muscle cells. In accordance with that finding, the higher serum MOTS-C levels in the current study were found to be associated with lower serum glucose levels. In the aforementioned study by Lee et al., it was also shown that the MOTS-C level in circulation decreased associated with increasing insulin resistance in mice and increasing age [15]. Therefore, MOTS-C is thought to be related to processes such as obesity, diabetes and ageing [13]. As improvement in insulin sensitivity has been obtained with intraperitoneal MOTS-C injection in animal studies, it has been postulated that MOTS-C could be used in the future in diabetes treatment and even in the complete recovery of diabetes [15, 17, 21]. Ramanjaneya et al. showed that the MOTS-C level was lower in cases with type 2 DM compared with a healthy control group [22]. However, no study could be found in the literature which has evaluated the MOTS-C level in GDM cases. The results of the current study demonstrated that the MOTS-C level was lower in GDM cases than in the healthy pregnant cases. Mitochondrial dysfunction has been suggested as a mechanism causing both type 2 DM and GDM, which is supported by the results of the current study [10, 23].

It is of great importance that GDM is screened and diagnosed during antenatal examinations as it is associated with perinatal complications. For both mother and infant,
there is an increased risk of metabolic disease in the future [8]. The oral glucose tolerance tests including the “One-step” approach with 75 g OGTT or the “Two-step” approach with a 50 g (non-fasting) screening followed by a 100 g OGTT for those who screen positive are applied in the 24–28th weeks of pregnancy for the diagnosis of GDM. The American Diabetes Association has stated that either the one-step or two-step approach can be used in GDM diagnosis [1]. Several organizations support the two-step approach for GDM diagnosis in pregnancy, while the one-step approach using a 75 g, 2 h OGTT has been used and promoted by other organizations such as the International Association of Diabetes and Pregnancy Study Group (IADPSG) [1, 3]. To date, there is no universally accepted standard approach to diagnose GDM. Furthermore, a 2017 Cochrane review supported that no specific screening approach has been demonstrated to be optimal [24].

Just as some healthcare institutions apply a universal screening approach for the determination of GDM, there are also opinions that it is only necessary to apply screening and diagnostic tests to those with risk factors [25, 26]. There is an increased risk of the development of GDM in patients with various risk factors such as a family history of DM, obesity, GDM diagnosed in a previous pregnancy, or a history of glucosuria. However, the positive predictive value of these risk factors is low and there may be no risk factor in 50 % of cases diagnosed with GDM [3, 8]. In addition, factors such as the need for fasting for the OGTT used in diagnosis, the taking of multiple blood samples, the need to wait at hospital until all the blood samples have been taken, and that the sugary water which has to be drunk can cause nausea and vomiting, decrease patient compliance with these tests and some patients refuse to have the tests [27].

For the reasons mentioned above, when GDM is not diagnosed in pregnancy and therefore the necessary treatments are not applied, this can cause adverse outcomes for both the mother and fetus. Biomarkers which can provide diagnosis by taking a single blood sample without applying OGTT, not only increase patient compliance but also reduce the workload of healthcare personnel. The properties expected of an ideal biomarker are that it should be low-cost, can be applied non-invasively, be reproducible, and have high diagnostic capacity for the differentiation of patients from healthy cases. Studies have been conducted showing that rather than OGTT, various biomarkers such as adiponectin, HbA1c, C-reactive protein, and glycosylated fibronectin, could

![ROC Curve](image)

**Figure 1:** ROC curve analysis of MOTS-C for the determination of gestational diabetes mellitus (area under curve: 0.762, 95 % confidence interval: 0.657–0.867).
be useful in diagnosis [28–30]. There has also been research of biomarkers such as single nucleotide polymorphisms, SNPs, DNA methylation, and miRNAs, as potential biomarkers to diagnose GDM [27]. However, none of these has proved to be an ideal biomarker which can be used alone in the diagnosis of GDM in clinical practice.

Aiming to increase the sensitivity and specificity of individual factors and to increase predictive power, a combination of several of these molecular biomarkers would most probably be the nearest to an ideal biomarker for GDM diagnosis.

The current study is of value as it shows for the first time in literature that MOTS-C seems to be a novel potential biomarker that could be used in the diagnosis of GDM.

When diagnosis is made at 24–28 weeks, there is limited time to improve the pregnancy outcomes. Therefore, if GDM diagnosis could be made earlier, there would be a longer period to be able to prevent pregnancy complications. Several studies have been made of biomarkers which could predict the patients who would develop GDM [31, 32]. The current study has laid the ground for further studies which could investigate whether or not MOTS-C could predict GDM in the early weeks of pregnancy.

Conclusions

The current study is the first in literature to have investigated the serum MOTS-C level in GDM, and the results showed that the serum MOTS-C level was lower in GDM patients than in the healthy pregnant women, supporting the view that mitochondrial dysfunction may have a role in the etiopathogenesis of GDM. It was also found that MOTS-C can be one of the future biomarkers to be used to diagnose GDM. The current study can be considered of value as the first to demonstrate that an increase in serum MOTS-C level is associated with a decrease in the serum glucose level in pregnant women. The results of this study could be of guidance for further studies which could investigate the use of MOTS-C in the treatment of GDM.

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Research ethics: The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethics committee approval number: # 08/2019.

Informed consent: Informed consent was obtained from all individuals included in this study, or their legal guardians or wards.

Author contributions: The authors have accepted responsibility for the entire content of this manuscript and approved its submission. Acquisition of data: Abdullah Tok. Analysis and interpretation of data for the work: Serdar Özer and Filiz Alkan Baylan. Drafting the work or revising it critically for important intellectual content: Serdar Özer. Competing interests: The authors state no conflict of interest. Research funding: None declared.

Data availability: The raw data can be obtained on request from the corresponding author.

References