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Original Research

Evaluation of MANF and MOTS-c Levels in Women With Gestational Diabetes Mellitus and Healthy Pregnant Women: A Prospective Cohort Study

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Abstract

Background: Gestational diabetes mellitus (GDM) is a common pregnancy complication characterized by metabolic stress and mitochondrial dysfunction, for which reliable circulating biomarkers are still lacking. This study aimed to investigate serum mesencephalic astrocyte-derived neurotrophic factor (MANF) and mitochondrial open reading frame of the 12S rRNA-c (MOTS-c) levels in women with GDM and to compare them with levels in healthy pregnant and non-pregnant women. We also sought to identify potential associations with metabolic and demographic parameters. **Methods**: This prospective cohort study was conducted from January 2025 to May 2025 and enrolled three groups: women with GDM, healthy pregnant women, and healthy non-pregnant women. Age, anthropometric data, and routine biochemical parameters, including fasting glucose, insulin, hemoglobin A1c (HbA1c), alanine aminotransferase (ALT), creatinine, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides, were assessed. Serum MANF and MOTS-c levels were also measured. **Results**: The GDM group was significantly older (33.2 $\pm$ 5.6 years) than the healthy pregnant (29.3 $\pm$ 4.5 years) and non-pregnant control groups (28.6 $\pm$ 5.2 years) ($p = 0.011$). MANF levels were significantly lower in pregnant women compared with controls ($p < 0.001$), whereas MOTS-c levels were significantly higher ($p < 0.001$). Receiver operating characteristic (ROC) analysis demonstrated limited discriminatory ability of MANF (area under the curve [AUC] = 0.567, $p = 0.301$) and MOTS-c (AUC = 0.604, $p = 0.111$) in distinguishing women with GDM from healthy pregnant women. Furthermore, within the GDM group, MANF and MOTS-c levels were not associated with age, height, weight, body mass index (BMI), waist circumference, glucose, insulin, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), lipid profile, or liver enzyme levels. **Conclusions**: This study shows that MOTS-c levels increase and MANF levels decrease during pregnancy, but neither is associated with GDM, indicating pregnancy-related metabolic changes rather than GDM-specific alterations. Although they are not useful for GDM diagnosis, these peptides may provide insight into novel metabolic pathways and hold therapeutic potential in high-risk pregnancies. **Study Registration**: The study has been registered on https://zenodo.org/ (registration link: https://doi.org/10.5281/zenodo.18776009).

Keywords: pregnancy; gestational diabetes; mitochondrial proteins; biomarkers; metabolism

1. Introduction

As a pregnancy-induced metabolic condition, gestational diabetes mellitus (GDM) represents one of the most prevalent complications of gestation, with incidence rates varying from 1% to 20% across global populations [1,2]. This wide variation is attributed to differences in diagnostic criteria and population characteristics [1,2]. GDM is associated with increased odds of maternal and fetal complications, including macrosomia, neonatal hypoglycemia, preeclampsia, and cesarean delivery [3]. Women diagnosed with GDM face a significantly higher likelihood of developing type 2 diabetes mellitus (T2DM) following delivery [4]. In a meta-analysis of observational studies, the cumulative incidence of type 2 diabetes over 10 years was 16% in women with gestational diabetes and 2% in women without gestational diabetes [5]. The pathogenesis of GDM is multifactorial. Recent studies suggest that molecular mechanisms such as endoplasmic reticulum stress and mitochondrial dysfunction may further contribute to GDM development [6,7].

Mesencephalic astrocyte-derived neurotrophic factor (MANF), initially identified as a protein supporting neuronal survival, has since been shown to be involved in pancreatic β-cell homeostasis [8,9]. MANF is secreted in response to endoplasmic reticulum stress and exerts protective effects against diabetes by inhibiting β-cell apoptosis

[8,9]. An experimental study demonstrates that MANF deficiency leads to severe diabetes, while MANF administration mitigates β-cell damage [10]. However, clinical findings are conflicting. Some studies report elevated plasma MANF levels in individuals with T2DM [8,9], whereas others associate high MANF with disease activity in children with type 1 diabetes mellitus (T1DM) [11].

Mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), a mitochondrial-encoded peptide derived from the 12S rRNA region, plays a significant role in modulating metabolic processes [12]. MOTS-c enhances insulin sensitivity and may be protective against obesity and T2DM. In animal models, MOTS-c was suggested to ameliorate fat diet-induced insulin resistance and obesity by stimulating the AMP-activated protein kinase (AMPK) pathway [12]. In humans, MOTS-c levels have been found to be lower among obese individuals and can increase with exercise [13]. Although several studies have observed lower circulating MOTS-c levels in GDM patients relative to normoglycemic pregnant controls [14,15], data on this association remains limited, and comprehensive analyses are lacking.

Limited data exist on the potential roles of MANF and MOTS-c in pregnancy and GDM. We aimed to compare serum MANF and MOTS-c levels among pregnant women with and without GDM and in healthy non-pregnant women of reproductive age.

2. Materials and Methods

2.1 Study Groups

This prospective cohort study was carried out at Konya City Hospital, Konya, Turkey, between January 2025 and May 2025. The study population comprised three groups: (1) pregnant women aged 18–35 years diagnosed with GDM at our Obstetrics and Gynecology Polyclinic; (2) reproductive-aged (18–35 years) non-GDM pregnant women; and (3) healthy, non-pregnant women of reproductive age (18–35 years) without active metabolic, inflammatory, or endocrine disorders, recruited from the Endocrinology and Diet Polyclinics—excluding those with body mass index (BMI) $<$18.5 or $>$30 kg/m^2.

2.2 Participant Selection

Exclusion criteria for pregnant women encompassed pre-pregnancy diabetes, twin or multiple pregnancies, complicated pregnancies [e.g., preeclampsia, eclampsia, hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome], thyroid dysfunction requiring medication, inflammatory diseases (e.g., systemic lupus erythematosus, Crohn's disease), cancer, active infections, or unavailability of laboratory data.

All pregnant women and the healthy control group were consecutively identified among individuals applied to Konya City Hospital. Gestational diabetes mellitus was diagnosed through standardized screening methodology utilizing the 75-gram oral glucose tolerance test, with diagnostic confirmation based on established plasma glucose values: fasting levels $\geq$92 mg/dL (5.1 mmol/L), one-hour post-glucose administration $\geq$180 mg/dL (10.0 mmol/L), and/or two-hour post-glucose administration $\geq$153 mg/dL (8.5 mmol/L), following the International Association of Diabetes and Pregnancy Study Groups guidelines, while ensuring appropriate gestational timing (beyond 28 weeks gestation) for accurate assessment [16].

2.3 Data Collection

2.3.1 Demographic and Anthropometric Data

Demographics, weight, height, and waist circumference were obtained. Weight and height were measured using calibrated clinical scales during hospital visits. Waist circumference was determined with a non-elastic tape at the midpoint between the lower rib and iliac crest. The BMI for each subject was determined using the equation BMI = weight (kg) / [height (m)]^2.

2.3.2 Blood Sample Collection and Routine Biochemical Analyses

Fasting blood samples (8–12 hours) were collected in serum separator tubes during routine clinical evaluations. After centrifugation (3000 rpm, 10 minutes), serum aliquots were stored at –80 °C. Residual samples from routine tests were used to measure MANF and MOTS-c levels to avoid additional blood draws.

Biochemical analyses included fasting glucose (mg/dL), hemoglobin A1c (HbA1c, %), insulin (µIU/mL), creatinine (mg/dL), alanine aminotransferase (ALT, U/L), triglycerides (mg/dL), high-density lipoprotein cholesterol (HDL-C, mg/dL), low-density lipoprotein cholesterol (LDL-C, mg/dL). All quantifications were performed using automated clinical chemistry analyzers (e.g., Roche Cobas®, Mannheim, Germany or Abbott Architect®, Abbott Park, IL, USA) in our biochemistry laboratory, which is an accredited laboratory. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was employed to quantify insulin resistance using the following formula: HOMA-IR = [fasting glucose (mg/dL) $\times$ fasting insulin (µIU/mL)] / 405. The triglyceride–glucose (TyG) index was calculated as: TyG = ln [fasting triglycerides (mg/dL) $\times$ fasting glucose (mg/dL) / 2]. The single point insulin sensitivity estimator (SPISE) index was calculated using the formula: SPISE = 600 $\times$ (HDL-C$^{0.185}$) / (triglycerides$^{0.2}$ $\times$ BMI$^{1.338}$) [17].

2.3.3 Assays for MANF and MOTS-c Measurement

Stored serum samples were thawed and analyzed for MANF and MOTS-c levels using commercial enzyme-linked immunosorbent assay (ELISA) kits (catalog no: ELK10529 and ELK7627, ELK Biotechnology, Wuhan, Hubei, China), following manufacturer protocols. Sample absorbance was quantified using a calibrated microplate

Table 1. Summary of demographics and laboratory measurements with regard to groups.

	Healthy controls (n = 29)	Healthy pregnant women (n = 40)	Gestational diabetes mellitus (n = 40)	*p*
Age, years	27 (24–32)	26 (23–31)	31 (27–35)$^{\#}$	**0.011**§
Weight, kg	61.0 (55.0–72.0)	73.0 (61.5–84.5)*	76.0 (66.5–88.0)*	**<0.001**§
Height, m	1.66 ± 0.07	1.61 ± 0.05*	1.59 ± 0.05*	**<0.001**†
Body mass index, kg/m^2	22.04 (20.70–24.68)	27.76 (23.83–33.69)*	30.37 (27.03–35.00)*	**<0.001**§
Waist circumference, cm	72 (68–82)	99 (93–111)*	105 (97–112)*	**<0.001**§
MANF, pg/mL	341.62 (241.79–423.44)	13.96 (7.89–34.65)*	10.04 (7.73–21.60)*	**<0.001**§
MOTS-c, ng/mL	52.67 (35.22–74.54)	137.80 (121.00–152.40)*	131.85 (116.60–142.95)*	**<0.001**§
HbA1c, %	5.15 ± 0.32	4.99 ± 0.34	5.48 ± 0.49*$^{\#}$	**<0.001**†
Glucose, mg/dL	84 (78–92)	77 (70–83)*	89.5 (81–100)$^{\#}$	**<0.001**§
Insulin, µIU/mL	7.28 (5.32–11.00)	9.85 (5.44–32.10)	10.30 (7.10–15.80)	0.095§
HOMA-IR	1.51 (1.16–2.23)	1.88 (1.02–6.51)	2.34 (1.24–3.89)	0.127§
TyG	7.87 (7.67–8.10)	8.9 (8.55–9.19)*	9.08 (8.85–9.35)*	**<0.001**§
SPISE	8.52 (7.03–9.62)	5.27 (3.92–6.99)*	4.47 (3.86–5.53)*	**<0.001**§
LDL-C, mg/dL	93.0 (82.0–105.0)	123.5 (104.5–142.5)*	123.5 (103.5–160.5)*	**<0.001**§
HDL-C, mg/dL	54.59 ± 11.75	65.13 ± 16.17*	57.83 ± 12.61	**0.006**†
Triglyceride, mg/dL	69 (52–83)	193 (139–241.5)*	225.5 (151–292)*	**<0.001**§
ALT, U/L	13.0 (10.0–15.0)	14.5 (12.0–17.5)	13.0 (9.0–17.0)	0.095§
Creatinine, mg/dL	0.67 (0.64–0.72)	0.48 (0.43–0.53)*	0.46 (0.42–0.54)*	**<0.001**§

Descriptive statistics are presented using mean ± standard deviation for normally distributed continuous variables, median (25th percentile–75th percentile) for non-normally distributed continuous variables and frequency (percentage) for categorical variables.

†One-way analysis of variance (ANOVA), *Significantly different from "Healthy controls"; $^{\#}$Significantly different from "Healthy pregnants"; §Kruskal-Wallis test. Statistically significant *p* values <0.05 are shown in bold.

Abbreviations: ALT, alanine aminotransferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; SPISE, Single Point Insulin Sensitivity Estimator; TyG, triglyceride–glucose index; LDL-C, low-density lipoprotein cholesterol; MANF, mesencephalic astrocyte-derived neurotrophic factor; GDM, gestational diabetes mellitus; BMI, body mass index; MOTS-c, mitochondrial open reading frame of the 12S rRNA-c.

reader, with analyte concentrations determined from standard curve regression analysis.

2.4 Statistical Analysis

All analyses were conducted using IBM SPSS version 27.0 (IBM Corp., Armonk, NY, USA), with a significance threshold of 5% alpha error (two-tailed; $p < 0.05$). The normality of continuous variables was assessed using the Shapiro-Wilk test. For normally distributed variables, parametric analyses were performed: one-way analysis of variance (ANOVA) for comparisons across >2 groups or Student's *t*-test for two-group comparisons. Non-normally distributed continuous variables were analyzed using the Kruskal-Wallis test. Pairwise comparisons were adjusted using Bonferroni correction. Diagnostic performance was assessed through receiver operating characteristic (ROC) curve analysis. Bivariate associations between continuous variables were examined using Spearman's rank-order correlation coefficients. To account for potential confounding by age, age-adjusted multivariable analyses were performed. Binary logistic regression models were constructed to evaluate whether MANF and MOTS-c were independently associated with pregnancy and GDM status after adjustment for age.

In addition, analysis of covariance (ANCOVA) was applied, when appropriate, to compare biomarker levels between groups while controlling for age as a covariate.

3. Results

Women in the GDM group (n = 40) were significantly older (33.2 ± 5.6 years) than both healthy pregnant women (29.3 ± 4.5 years; n = 40) and the control group (28.6 ± 5.2 years; n = 29) ($p = 0.011$). All pregnant women (both healthy and GDM) had significantly higher body weight, BMI, and waist circumference compared to the control group ($p < 0.001$) (Table 1). While MANF levels were significantly lower in pregnant women compared to controls ($p < 0.001$), MOTS-c levels were significantly higher ($p < 0.001$). As anticipated, the GDM group had significantly higher HbA1c and fasting glucose levels relative to both other groups ($p < 0.001$). Lipid profiles revealed higher HDL-C among healthy pregnant women compared to controls ($p = 0.006$). Additionally, LDL-C and triglyceride levels were significantly elevated in both pregnant groups compared to controls ($p < 0.001$). Significant differences in insulin resistance surrogate indices were observed among the three study groups for the TyG and SPISE indices (both $p < 0.001$), whereas HOMA-IR did not differ significantly across groups ($p = 0.127$).

The TyG was lowest in healthy controls 7.87 and significantly higher in both healthy pregnant women 8.90 and women with GDM 9.08 (overall $p < 0.001$). Post-hoc analyses showed that TyG values were significantly elevated in both pregnant groups compared with non-pregnant controls (both $p < 0.001$), while no significant difference was observed between women with GDM and healthy pregnant women ($p > 0.05$).

Similarly, the SPISE index differed significantly among groups (overall $p < 0.001$). SPISE values were highest in healthy controls 8.52 and significantly lower in healthy pregnant women 5.27 and women with GDM 4.47. Post-hoc comparisons confirmed significantly reduced SPISE levels in both pregnant groups compared with controls (both $p < 0.001$), with no significant difference between the GDM and healthy pregnant groups ($p > 0.05$).

In contrast, HOMA-IR showed a stepwise numerical increase from healthy controls 1.51 to healthy pregnant women 1.88 and women with GDM 2.34, but this trend did not reach statistical significance ($p = 0.127$) (Table 1).

The ability of MANF to differentiate between GDM and healthy pregnant women showed an area under the curve (AUC) of 0.567 (95% CI: 0.441–0.694), which was not statistically significant ($p = 0.301$). Similarly, MOTS-c demonstrated an AUC of 0.604 (95% CI: 0.479–0.729) without significance ($p = 0.111$) (Fig. 1, Table 2). These low AUC values suggest that MANF and MOTS-c have limited discriminatory performance and are not reliable diagnostic biomarkers for GDM.

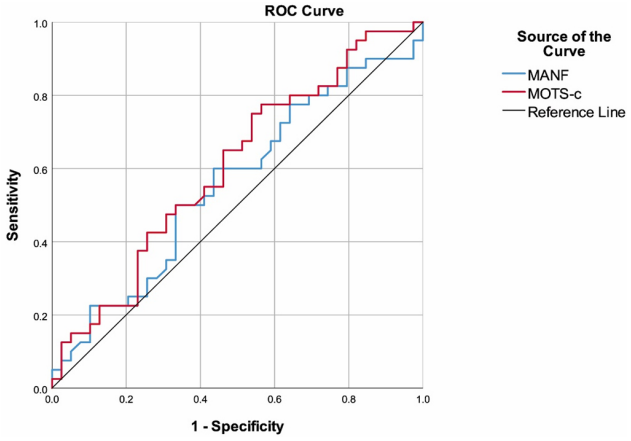

Fig. 1. Receiver operating characteristic (ROC) curves of MANF and MOTS-c for discriminating gestational diabetes mellitus from healthy pregnant women. ROC analyses were performed using GDM as the positive state variable. The diagonal line represents the line of no discrimination (AUC = 0.5). Corresponding AUC values with 95% confidence intervals are presented in Table 2.

We performed correlation analyses within the GDM group to evaluate associations between MANF and MOTS-c levels and other clinical characteristics, and the resulting correlation patterns are presented numerically in Table 3 and graphically in a heatmap (Fig. 2). None of the analyses showed any relationships, including weight, height, BMI, waist circumference, HbA1c, glucose, insulin, HOMA-IR, TyG, SPISE, LDL-C, HDL-C, triglycerides, ALT and creatinine.

Table 2. Performance of MANF and MOTS-c to discriminate between gestational diabetes mellitus and healthy pregnant women, ROC curve analysis.

	AUC (95% CI)	*p*
MANF	0.567 (0.441–0.694)	0.301
MOTS-c	0.604 (0.479–0.729)	0.111

Abbreviations: AUC, area under the curve.

Binary logistic regression analyses were performed to evaluate whether MOTS-c was independently associated with pregnancy and GDM after adjustment for age. In the first model (pregnant vs. non-pregnant women), MOTS-c remained an independent predictor of pregnancy (OR = 1.091, 95% CI: 1.045–1.139, $p < 0.001$), whereas age was not significant ($p = 0.981$). The model correctly classified 94.1% of participants. In the second model (GDM vs. healthy pregnant women), MOTS-c was also independently associated with GDM (OR = 1.093, 95% CI: 1.048–1.140, $p < 0.001$), while age showed no statistically significant effect ($p = 0.055$). The model achieved an overall classification accuracy of 92.8%. These findings indicate that the association between MOTS-c and both pregnancy and GDM is independent of age. MANF did not show an independent association with either pregnancy or GDM in age-adjusted models ($p > 0.05$).

4. Discussion

Our findings demonstrating significant differences in TyG and SPISE across study groups are consistent with previous reports showing that these indices correlate with HOMA-IR as surrogate markers of insulin sensitivity. In line with our results, Sağnıç *et al.* [18] reported that although the TyG index differed between pregnant and non-pregnant women, its diagnostic performance in distinguishing GDM from normoglycemic pregnancies was limited, suggesting that TyG mainly reflects physiological pregnancy-related insulin resistance rather than GDM-specific metabolic dysfunction. Similarly, in our cohort, both indices showed low sensitivity for GDM, indicating limited utility as stand-alone screening tools but potential value for excluding adverse metabolic profiles. Although SPISE has been shown to predict cardiovascular outcomes in type 2 diabetes and proposed for population screening [19,20], we observed significantly lower SPISE values in pregnant women regardless of GDM status, implying that it captures gestational metabolic adaptation rather than GDM itself. In contrast, HOMA-IR did not differ between groups,

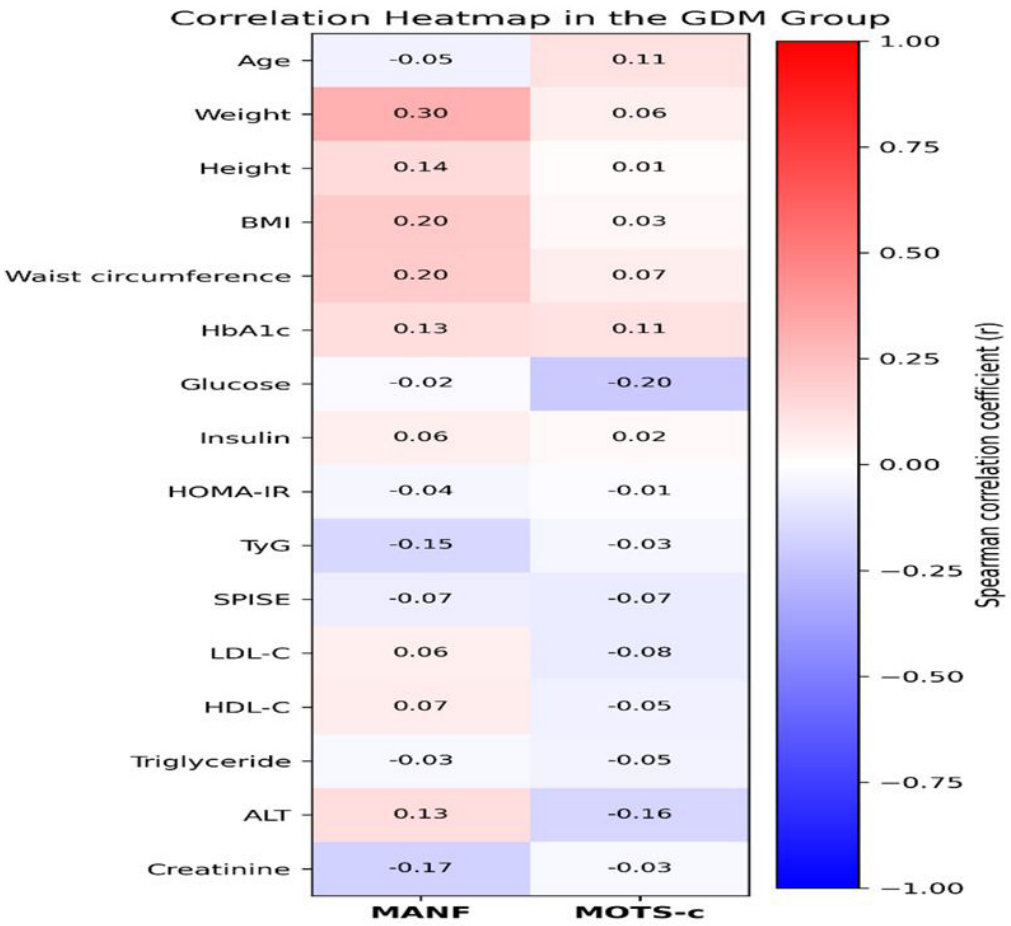

Fig. 2. Heatmap representation of Spearman correlation coefficients between circulating MANF and MOTS-c levels and clinical/metabolic parameters in the GDM group. Each cell displays the correlation coefficient (r), rounded to two decimal places for visualization purposes; exact numerical values are provided in Table 3. The color scale ranges from blue (negative correlation) to red (positive correlation), with white indicating weak or no correlation. Overall, correlations between biomarkers and metabolic variables were weak.

supporting previous evidence that insulin-based indices are constrained in pregnancy due to physiological hyperinsulinemia and hormonal effects [21].

Our findings revealed that, irrespective of GDM status, pregnant women exhibited significantly lower MANF and higher MOTS-c levels compared with non-pregnant controls, while neither biomarker discriminated between GDM and normoglycemic pregnancies. No significant correlations were observed between MANF/MOTS-c and demographic, anthropometric, or metabolic parameters in women with GDM.

MOTS-c is known to enhance insulin sensitivity and protect against obesity and insulin resistance in experimental models [12,22], and reduced circulating levels have been reported in obesity, insulin resistance, and both type 1 and type 2 diabetes [23–25]. Exercise-induced MOTS-c expression further supports its role in metabolic regulation [26]. An experimental study has also demonstrated protective effects of MOTS-c in autoimmune diabetes through modulation of T-cell function and mTORC1 signaling [13].

Despite this established metabolic role, the involvement of MOTS-c in GDM remains unclear. In our study, MOTS-c levels were significantly higher in both GDM and non-GDM pregnancies compared with non-pregnant controls; however, ROC analysis demonstrated limited discriminatory ability for GDM (AUC = 0.604, 95% CI crossing 0.50), indicating that MOTS-c cannot serve as a reliable standalone diagnostic biomarker. Furthermore, MOTS-c levels were not associated with any metabolic indices in women with GDM.

In contrast to our findings, previous Turkish and Chinese studies reported significantly lower MOTS-c levels in GDM, with inverse correlations to glucose and obesity-related parameters [14,15]. This discrepancy may reflect differences in gestational timing, population characteristics, or threshold-dependent regulatory mechanisms. However, our findings indicate that MOTS-c levels are closely linked to the physiological state of pregnancy rather than GDM-specific pathology, suggesting a potential role in gestational metabolic adaptation, possibly as a compensatory response

Table 3. Correlations between MANF, MOTS-c and other variables.

		Gestational diabetes mellitus (n = 40)	
		MANF	MOTS-c
MANF	r	-	–0.184
	p	-	0.254
Age	r	–0.052	0.114
	p	0.750	0.482
Weight	r	0.305	0.055
	p	0.056	0.736
Height	r	0.140	0.014
	p	0.390	0.930
Body mass index	r	0.205	0.025
	p	0.204	0.877
Waist circumference	r	0.200	0.066
	p	0.228	0.694
HbA1c	r	0.127	0.114
	p	0.436	0.485
Glucose	r	–0.017	–0.205
	p	0.919	0.216
Insulin	r	0.062	0.016
	p	0.709	0.921
HOMA-IR	r	–0.035	–0.009
	p	0.836	0.956
TyG	r	–0.152	–0.034
	p	0.363	0.837
SPISE	r	–0.065	–0.073
	p	0.688	0.656
LDL-C	r	0.062	–0.077
	p	0.705	0.635
HDL-C	r	0.072	–0.049
	p	0.659	0.766
Triglyceride	r	–0.030	–0.046
	p	0.855	0.777
ALT	r	0.131	–0.159
	p	0.421	0.328
Creatinine	r	–0.174	–0.025
	p	0.283	0.877

Abbreviations: r, Spearman correlation coefficient.

to pregnancy-induced metabolic stress [27,28]. In this respect, our study contributes to the existing literature by characterizing baseline MOTS-c patterns in normal pregnancy and provides a contextual framework for interpreting MOTS-c alterations observed in GDM. Although MOTS-c has demonstrated therapeutic potential in experimental models [14], its limited diagnostic utility in our cohort parallels the constraints reported for other proposed biomarkers [29].

MANF, a protein critical in endoplasmic reticulum stress response and metabolic regulation, has demonstrated protective effects against β-cell damage and insulin resistance in preclinical studies [9,11,30]. In a cross-sectional study, circulating MANF levels were significantly decreased in T2DM patients compared to both overweight/obese individuals and healthy controls. In that cohort, plasma MANF was negatively correlated with fasting blood glucose and osteopontin, and positively correlated with HDL-C. Multivariable regression identified osteopontin, HDL-C and BMI as independent predictors of MANF levels. Moreover, trend analysis showed that lower MANF carried higher relative risk for T2DM [8]. In contrast, other human studies report increased plasma MANF in newly diagnosed prediabetes and in children with T1DM [9,30]. To our knowledge, no prior study has compared MANF levels between pregnant and non-pregnant women or between GDM and non-GDM pregnancies. In the current study, we found that plasma MANF was significantly lower in all pregnant women versus healthy non-pregnant controls; however, MANF did not differ between GDM and non-GDM pregnancies. Consistent with this observation, MANF did not show an independent association with either pregnancy or GDM in age-adjusted regression models. The observed decrease in MANF during pregnancy may reflect inadequate adaptation to gestational metabolic demands, particularly endoplasmic reticulum stress induced by placental hormones and insulin resistance. While MANF is known to enhance β-cell survival and insulin sensitivity in non-pregnant models [30], its reduced levels in pregnancy suggest a unique regulatory mechanism. These may be associated with hormonal shifts (e.g., elevated placental lactogen, estrogen) or physiological insulin resistance during gestation, potentially acting to suppress MANF expression. Although MANF does not serve as a biomarker for GDM, its pregnancy-specific downregulation suggests a potential role in pregnancy-related metabolic adaptation.

Although our study fills an important gap in knowledge, the single-center design and small sample size may restrict the generalizability of the findings, particularly given the heterogeneous nature of metabolic adaptations in pregnancy. The inclusion of non-pregnant controls and comparison of pregnant women with and without GDM adds considerable value, but the smaller size of this group may reduce statistical power (considering that subtle changes may be critical in GDM pathophysiology). Moreover, our sampling strategy may have introduced selection bias. For instance, the age demonstrated a significant difference between groups, with the GDM group being significantly older than the other two. The longitudinal tracking of MOTS-c and MANF levels from pre-pregnancy through postpartum periods would provide much-needed data to this topic. The lack of mechanistic data (e.g., tissue-specific expression, endoplasmic reticulum stress markers) further limits our ability to interpret the functional significance of changes in MOTS-c/MANF. Future multicenter studies with serial measurements are needed to validate these preliminary observations and explore underlying pathways.

Limitations

This study has several limitations. First, detailed dietary intake and physical activity levels were not systematically assessed. Given that both nutritional status and exercise have been shown to influence metabolic pathways and stress-responsive peptides, including MANF and MOTS-c, unmeasured variability in these factors may have affected circulating biomarker levels. Second, the markedly lower MANF concentrations observed in pregnant women may, at least in part, reflect physiological plasma volume expansion during pregnancy, which can lead to dilution of circulating proteins. As hematocrit or plasma volume markers were not available for adjustment, a dilutional effect cannot be excluded. Finally, the relatively modest sample size within each group may have limited the statistical power to detect subtle between-group differences, particularly between women with GDM and healthy pregnant controls. Accordingly, our findings should be interpreted with caution and warrant confirmation in larger, prospectively designed cohorts incorporating comprehensive lifestyle assessments. Additionally, the relatively low AUC values observed in the ROC analyses indicate limited diagnostic discrimination of these biomarkers for GDM, which should be considered when interpreting their clinical applicability.

5. Conclusions

This study demonstrates pregnancy-associated changes in circulating MOTS-c and MANF levels and indicates that GDM itself does not exert an additional effect on these parameters. In addition, TyG and SPISE indices differed significantly between pregnant and non-pregnant women but showed limited sensitivity for GDM, supporting the notion that these indices primarily reflect physiological gestational insulin resistance rather than GDM-specific metabolic dysfunction. Despite statistically significant group differences and independent associations observed in regression models, neither MANF nor MOTS-c exhibited sufficient discriminatory power for GDM, with AUC values close to chance. This apparent discrepancy highlights the distinction between statistical association and diagnostic performance, whereby a biomarker may be related to disease risk yet lack adequate classification accuracy. Accordingly, MANF and MOTS-c are unlikely to serve as standalone diagnostic biomarkers for GDM, although they may provide insight into pregnancy-related metabolic adaptation. Future confirmation of these findings will require larger, well-characterized human cohorts, including appropriately matched control groups and longitudinal assessment of MOTS-c and MANF across gestation.

Availability of Data and Materials

The anonymized dataset generated during this study is publicly available in the Zenodo repository at https://doi.org/10.5281/zenodo.18776009 in accordance with the journal's data sharing policy.

Author Contributions

HÇB: writing – review & editing, methodology, formal analysis, data curation, conceptualization. ÜC: formal analysis, data curation. MK: data curation. NGK: formal analysis, data curation. OG: formal analysis, data curation. OA: writing – review & editing, methodology, formal analysis, conceptualization. All authors contributed to critical revision of the manuscript for important intellectual content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study adhered to the ethical principles of the Helsinki Declaration and received approval from the Non-Interventional Clinical Research Ethics Committee of Konya City Hospital (Approval number: 2025/34). Written informed consent was obtained from all participants prior to sample collection. Residual blood samples were anonymized to protect participant confidentiality, and analyses were conducted using de-identified data.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG47924.

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