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Original Research

The Role of Follistatin-Like 1 Levels and Follistatin-Like 1 Gene Polymorphism in the Development of Gestational Diabetes Mellitus

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Abstract

Background: Gestational diabetes mellitus (GDM) is characterized by increased insulin resistance that develops during pregnancy and is associated with adverse maternal and neonatal outcomes. Follistatin-like 1 (FSTL1) is a glycoprotein implicated in inflammatory pathways and mechanisms of insulin resistance. This study aimed to evaluate circulating FSTL1 levels and assess the potential association of *FSTL1* gene polymorphisms, rs12173 and rs869247, with GDM using molecular techniques. **Methods:** This retrospective case-control study included women diagnosed with GDM and healthy pregnant controls. Participants were enrolled after diagnosis, and clinical and laboratory data were analyzed. Genotyping of FSTL1 variants was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, while serum FSTL1 concentrations were quantified using the enzyme-linked immunosorbent assay (ELISA) method. **Results:** Serum FSTL1 levels did not differ significantly between patients with GDM and healthy controls ($p = 0.917$). No significant differences were observed in the distribution of genotypes or allele frequencies of the FSTL1 rs12173 and rs869247 variants between the groups ($p > 0.05$ for all). Serum triglyceride (TG) levels were significantly higher in individuals with the CT genotype of the rs12173 polymorphism compared with those with other genotypes [CT vs. CC, $p = 0.041$; CT vs. TT, $p = 0.018$] among patients with GDM. However, this association lost its statistical significance when a multivariable linear regression model was applied, adjusting for maternal age, body mass index (BMI), and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) (adjusted $p = 0.386$, 95% confidence interval [CI]: –0.038 to 0.098). No significant differences were observed in metabolic parameters, including fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), lipid profile, and HOMA-IR values, among the genotypes of the rs869247 polymorphism in patients with GDM ($p > 0.05$ for all). **Conclusions:** No significant association was observed between the FSTL1 gene variants rs869247 and rs12173 or serum FSTL1 levels and the risk of developing GDM.

Keywords: FSTL1; gene polymorphism; gestational diabetes mellitus; hypertriglyceridemia; insulin resistance

1. Introduction

Gestational diabetes mellitus (GDM) refers to impaired glucose metabolism first identified during pregnancy that may or may not resolve following delivery [1]. The global prevalence of GDM ranges from 1% to 28%. GDM is characterized by impaired glucose tolerance due to maternal pancreatic beta-cell dysfunction, leading to insufficient insulin production to regulate glucose homeostasis during pregnancy. In GDM, there is an increased risk of both short-term perinatal morbidity [2] and long-term cardiometabolic complications, such as maternal cardiometabolic disease [3] and childhood endocrine morbidity, such as diabetes mellitus and obesity [4]. As such, understanding the pathogenesis of GDM is crucial for preventing pregnancy-related complications.

Follistatin (FST) and follistatin-like (FSTL) genes belong to a large family of secreted protein acidic and rich in cysteine (SPARC) proteins that share structural and functional similarities. Five types of FSTL proteins have been identified, namely FSTL1, insulin-like growth factor binding protein 7 (IGFBP7 [FSTL-2]), FSTL4, FSTL3, and FSTL5. These proteins are expressed in nearly all tissues. FST and FSTL genes function by antagonising activins, which are members of the transforming growth factor-beta (TGF-β) superfamily involved in diverse cellular processes, such as cell proliferation and differentiation, fibrosis, tissue repair, and inflammatory responses. FSTL1 is a recently identified mediator, and data regarding its metabolic role remain limited [5].

Recent studies have demonstrated that multiple tissues, including adipose tissue, skeletal muscle, and the heart, secrete FSTL1. Therefore, FSTL1 functions as both a myokine and an adipokine. Comprehensive reviews have increasingly underscored FSTL1's broad significance as a

promising therapeutic target and an emerging biomarker across various inflammatory conditions [6]. Furthermore, its emerging role as an active adipokine highlights its significant regulatory functions in cardiovascular complications, including heart failure [7]. FSTL1, also known as transforming growth factor-β1-stimulated clone 36 (TSC36), has been demonstrated to play a role in insulin resistance. In skeletal muscle cells, FSTL1 stimulates muscle glucose uptake via adenosine monophosphate (AMP)-activated protein kinase activation [8]. Numerous studies have been conducted on FSTL1, particularly in relation to pathological processes associated with metabolic diseases [9,10,11]. In a recent study, patients with newly diagnosed metabolic syndrome exhibited substantially higher serum FSTL1 levels than controls, and FSTL1 levels were found to be positively correlated with fasting plasma glucose (FPG) [9]. A previous study examining the association between FSTL1 and insulin resistance reported that serum FSTL1 concentrations were significantly higher in patients with type 2 diabetes than in nondiabetic individuals [10]. In contrast, another study reported lower FSTL1 levels in patients with diabetes than in those with prediabetes, demonstrating the unclear role of FSTL1 in glycemic metabolic impairment [12]. These findings suggest that FSTL1 may function as a metabolic regulator and could serve as a potential biomarker in the pathogenesis of insulin resistance.

Currently, there is no published evidence regarding the potential impact of FSTL1 gene polymorphisms on the development of GDM. Therefore, the present study aimed to assess serum FSTL1 levels and investigate the potential role of FSTL1 gene polymorphisms rs869247 and rs12173 in the development of GDM. The FSTL1 rs869247 and rs12173 polymorphisms were selected based on the reported roles of the FSTL1 gene in metabolic diseases and the allele frequencies available in the NCBI SNP database (https://www.ncbi.nlm.nih.gov/snp/). This selection was further supported using the Varsome (https://varsome.com/) tool.

2. Materials and Methods

2.1 Study Population

This retrospective case-control study included women diagnosed with GDM and healthy pregnant controls. Participants were enrolled after diagnosis, and clinical and laboratory data were analyzed. Patients at high risk for GDM, such as patients with body mass index (BMI) >30 kg/m^2, a history of pregestational diabetes mellitus or GDM, polycystic ovary syndrome (PCOS), poor obstetric history, age ≥40 years, or a family history of diabetes mellitus in the first-degree relatives, were excluded from the study. Demographic data, anthropometric parameters (including age, weight, height, and BMI), and blood pressure measurements were recorded for all participants. FPG, fasting insulin levels, and lipid profile parameters, including total cholesterol, triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), were analysed. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to calculate the insulin resistance using the formula: (FPG in mg/dL × fasting insulin in μIU/mL) / 405. To screen for GDM, all participants underwent a 75 g oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation. GDM was diagnosed if any of the following criteria were met: FPG ≥92 mg/dL, 1-hour glucose value ≥180 mg/dL, or 2-hour glucose value ≥153 mg/dL after glucose loading. The control group comprised 79 healthy pregnant women who exhibited normal glucose tolerance based on OGTT results. The study was conducted between January and December 2022. The study protocol was approved by the Medical Ethics Committee of the Faculty of Medicine of Mugla Sitki Kocman University (Approval No: 13/II), and written informed consent was obtained from all participants prior to enrollment.

2.2 Molecular Analyses

After an overnight 12-h fast, blood samples were collected from the GDM patients between 24 and 28 weeks of gestation. Samples collected in ethylenediamine tetraacetic acid (EDTA) tubes for routine testing were stored at –40 °C until DNA extraction and genotyping. Genomic DNA was isolated from venous blood using the Hibrigen Blood DNA Isolation Kit (Cat. No: MG-KDNA-02-250; Hibrigen Biotechnology, Kocaeli, Turkey). The FSTL1 rs869247 and rs12173 polymorphisms were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR amplification was performed in a 25 μL reaction volume containing 100 ng of DNA using Thermo Scientific DreamTaq DNA Polymerase (Cat. No: EP0703, Thermo Fisher Scientific, Waltham, MA, USA) on a thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA). The PCR cycling conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. Primer sequences, annealing temperatures, and expected PCR product sizes are detailed in Table 1a. Subsequently, PCR products were digested with the restriction enzymes DdeI for rs12173 and BsaAI for rs869247 under the conditions specified in Table 1b. The digested fragments were separated by electrophoresis on a 2.5% agarose gel. Complete digestion was confirmed visually by at least two independent investigators.

2.3 Enzyme-Linked Immunosorbent Assay (ELISA) Quantification

After an overnight fast, blood samples were collected in EDTA tubes (for genetic analysis) and plain biochemistry tubes. The serum obtained from the plain tubes was centrifuged and stored at –40 °C until FSTL1 analysis. Serum FSTL1 levels were determined using the Human Follistatin-

Table 1. PCR-RFLP conditions for *FSTL1* rs869247 and rs12173 polymorphisms.

(a) PCR conditions used for the polymorphisms of the FSTL1 gene

Gene	Polymorphism	Primers	Temperature of annealing	Product size
FSTL1	rs12173	F: 5′-TGCTGTGCTGAGAGGGAATTT-3′ R: 5′-CCATGCTGCGAGGAACCTAT-3′	60 °C	249 bp
	rs869247	F: 5′-TGCAGCAGTAACAAGCAAACC-3′ R: 5′-GTCATGCTGCGAGGAACCTA-3′	60 °C	293 bp

(b) Restriction enzymes, digestion conditions, and restriction fragment sizes

Gene	Polymorphism	Restriction enzyme	Digestion conditions	Restriction fragment sizes
FSTL1	rs12173	*Dde*I	37 °C, 3 h	C allele: 249 bp T allele: 134 bp, 115 bp
	rs869247	*Bsa*AI	37 °C, 3 h	C allele: 293 bp T allele: 199 bp, 94 bp

PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; *FSTL1*, follistatin-like 1.

Like Protein 1 ELISA kit (Cat. No: E2702Hu, Bioassay Technology Laboratory/BT-LAB, Shanghai, China) according to the manufacturer's instructions. As per the manufacturer's one-step incubation protocol, 40 µL of serum samples were added to each well, followed immediately by the addition of 10 µL of anti-FSTL1 antibody and 50 µL of streptavidin-horseradish peroxidase (HRP). The plate was covered and incubated for 60 min at 37 °C. Following this single incubation step, the plate was washed five times with the provided wash buffer. Subsequently, 50 µL of substrate solution A and 50 µL of substrate solution B were added to each well, followed by incubation for 10 min at 37 °C in the dark. Finally, 50 µL of stop solution was added to terminate the reaction, and the absorbance was measured immediately at 450 nm [13].

2.4 Statistical Analyses

IBM SPSS Statistics for Windows v. 22.0 (IBM Corp., Armonk, NY, USA) was utilized for statistical analysis. The significance of observed genotype frequencies was assessed according to the Hardy-Weinberg principle by comparing observed and expected genotype frequencies. The Kolmogorov-Smirnov test and coefficient of variation were used to assess the normality of continuous variables. The Chi-square test was used to evaluate differences in categorical variables between groups. Adjusted odds ratio (OR) for genotypes were calculated using multivariable logistic regression models for age and BMI. The one-way analysis of variance (ANOVA), independent sample *t*-test, Mann-Whitney U test, and Kruskal-Wallis test were used to determine the statistical difference between the groups regarding continuous variables. Normally distributed variables were presented as mean ± standard deviation (SD), whereas for variables that were not normally distributed, descriptive statistics were presented as median (interquartile range, IQR). Potential outliers were evaluated using boxplot visualization and the IQR method (Q1 – 1.5 × IQR; Q3 + 1.5 × IQR). In addition, sensitivity (robustness) analyses were performed by repeating the analyses after excluding outliers, confirming that the results did not change substantially. Linear regression was used to evaluate the relationship between FSTL-1 levels and metabolic parameters. For variables that did not follow a normal distribution, such as serum TG levels, log-transformation was applied before regression analysis to satisfy normality assumptions. Furthermore, multivariable linear regression analysis was employed to evaluate the independent association between the rs12173 polymorphism and TG levels, adjusting for potential confounding factors, including maternal age, BMI, and HOMA-IR. A p-value of <0.05 was considered statistically significant. In addition, the sample size was determined using G-Power version 3.1.9.4 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The sample size was estimated based on an independent samples t-test to determine whether a statistically significant difference in FSTL1 levels existed between GDM patients and the control group. In the sample size calculation, the two-sided type I error rate (α) was set at 0.05 and the statistical power at 0.90. Assuming a 1:1 allocation ratio between the study and control groups, the standardized effect size was calculated as 0.5839818. Accordingly, a minimum total sample size of at least 126 people was determined.

3. Results

3.1 Basal Demographic Characteristics of the Participants

Table 2 summarizes the biochemical and demographic characteristics of women with GDM and healthy pregnant controls included in the study. The mean ages of the GDM (29.5 ± 4.6 years) and control groups (28.3 ± 4.5 years) were comparable. The GDM group exhibited significantly higher BMI, FPG, HOMA-IR, and Hemoglobin A1c (HbA1c) values than the control group ($p < 0.001$, $p <$

Table 2. Biochemical and demographic characteristics of GDM and control groups.

Variables	GDM (n = 72)	Control (n = 79)	*p*-value
Age (years)	29.5 ± 4.6	28.3 ± 4.5	0.085*
BMI (kg/m^2)	27.6 (25.6–29.8)	25.1 (23.4–27.9)	**<0.001****
FPG (mg/dL)	86.0 (78.0–93.8)	75.0 (71.0–80.0)	**<0.001****
Fasting Insulin (uIU/mL)	10.5 (6.9–15.5)	9.0 (5.9–12.0)	0.102**
HOMA-IR	2.2 (1.4–3.3)	1.6 (1.1–2.3)	**0.006****
HbA1c (%)	5.1 ± 0.4	4.9 ± 0.3	**0.002***
Total cholesterol (mg/dL)	248.1 ± 40.9	241.3± 47.9	0.372*
TGs (mg/dL)	203.0 (154.8–271.5)	187.5 (151.8–220.3)	0.129**
LDL (mg/dL)	131.9 ± 34.1	133.62 ± 36.8	0.781*
HDL (mg/dL)	71.7 ± 16.0	72.4 ± 14.2	0.790*
FSTL1 (ng/mL)	195.7 (142.3–369.2)	171.7 (128.8–832.7)	0.917**

*Mean ± standard deviation (SD); **Median (interquartile range, IQR). Bold values indicate statistical significance ($p < 0.05$).

GDM, gestational diabetes mellitus; n, number; BMI, body mass index; FPG, fasting plasma glucose; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HbA1c, hemoglobin A1c; TGs, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

0.001, $p = 0.006$, $p = 0.002$, respectively). No statistically significant difference in FSTL1 levels was observed between the groups (195.7 [142.3–369.2 ng/mL] in the GDM group vs. 171.7 [128.8–832.7] ng/mL in the control group; $p = 0.917$).

3.2 Associations of FSTL1 Genotypes With GDM

All observed genotype frequencies for both the rs12173 and rs869247 polymorphisms in both the GDM and control groups were consistent with the Hardy-Weinberg equilibrium ($p > 0.05$ for all). The genotype distributions and allele frequencies of the FSTL1 rs869247 and rs12173 polymorphisms were compared between the GDM and control groups. Regarding the FSTL1 gene rs12173 polymorphism, no statistically significant differences were observed in genotype or allele frequencies between the GDM and control groups ($p = 0.197$ and $p = 0.086$, respectively; Table 3). Similarly, genotype and allele distributions of the rs869247 polymorphism did not differ significantly between the two groups ($p = 0.264$ and $p = 0.627$, respectively; Table 3).

3.3 Associations of FSTL1 Genotypes With Metabolic Parameters in Patients With GDM

No significant differences were observed in metabolic parameters, including FPG, HbA1c, lipid profile, and HOMA-IR values, among the genotypes of the rs12173 polymorphism in patients with GDM ($p > 0.05$; Table 4a), except for serum TG levels in the unadjusted analysis. Initially, serum TG levels were significantly higher in individuals with the CT genotype of the rs12173 polymorphism compared with those with other genotypes (CT vs. CC, $p = 0.041$ and CT vs. TT, $p = 0.018$; Table 4a). Analysis of covariance (ANCOVA) indicated a significant difference in log-transformed TG levels among the genotypes ($F(2, 48) = 4.82$, $p = 0.012$). Some patients were excluded from this specific analysis due to missing data for the covariates. Post hoc analyses showed that individuals carrying the CT genotype had significantly higher log-transformed TG levels than those with the CC genotype ($p = 0.012$, Bonferroni-corrected). However, when a multivariable linear regression model was applied, adjusting for maternal age, BMI, and HOMA-IR using log-transformed TG levels, this association lost its statistical significance (adjusted $p = 0.386$, 95% confidence interval [CI]: –0.038 to 0.098). The results indicated that the observed variation in TGs was primarily influenced by BMI and insulin resistance rather than the genetic variant itself. There was no statistical difference in metabolic parameters among the genotypes of the rs869247 polymorphism in patients with GDM ($p > 0.05$ for all; Table 4b).

4. Discussion

The presence of diverse environmental and genetic risk factors may influence susceptibility to GDM. However, the underlying molecular mechanisms remain unclear. Improved understanding of the pathophysiological mechanisms involved may enhance the effectiveness of screening, facilitate timely intervention, and even support the development of preventive strategies. Herein, serum FSTL1 levels and FSTL1 gene polymorphisms rs869247 and rs12173 were not significantly associated with the development of GDM.

A strong correlation between BMI and GDM has been well established [14]. Fong et al. [15] reported that HbA1c is an essential risk factor for the development of GDM. Consistent with previous studies [16,17], the GDM group in our study exhibited significantly higher BMI, FPG, HbA1c

Table 3. Genotype and allele frequencies of the FSTL1 gene polymorphisms in the GDM and control groups.

FSTL1	GDM (n = 72) n (%)	Control (n = 79) n (%)	*p*-value	Adjusted OR (95% CI)
Genotype (rs12173)				
CC	11 (15.3)	9 (11.4)		1.0 (Ref.)
CT	22 (30.6)	16 (20.3)	0.197	0.53 (0.20–1.39)
TT	39 (54.2)	54 (68.4)		0.81 (0.24–2.68)
Allele (rs12173)				
C	44 (29.9)	34 (21.4)	0.086	
T	100 (70.1)	124 (78.6)		
Genotype (rs869247)				
CC	24 (33.3)	19 (24.1)		1.0 (Ref.)
CT	38 (52.8)	52 (65.8)	0.264	1.41 (0.63–3.16)
TT	10 (13.9)	8 (10.1)		1.77 (0.62–5.01)
Allele (rs869247)				
C	86 (59.7)	90 (57.0)	0.627	
T	58 (40.3)	68 (43.0)		

Chi-Square Test.

Adjusted odds ratio (OR) values were calculated using multivariable logistic regression models for genotypes, adjusted for age and BMI. Allelic frequencies were analyzed using unadjusted chi-square tests.

Table 4. Associations between metabolic parameters and the rs12173 and rs869247 polymorphisms in patients with GDM.

(A) Comparison of metabolic parameters among the rs12173 genotypes in the GDM group				
Variables	CC (n = 11)	CT (n = 22)	TT (n = 39)	*p*-value
FPG (mg/dL)	89.0 (85.0–98.0)	83.0 (76.8–90.8)	85.0 (77.0–93.0)	0.383**
Fasting Insulin (uIU/mL)	10.5 (7.2–15.3)	10.6 (8.2–19.4)	8.2 (6.4–13.7)	0.096**
HOMA-IR	2.1 (1.6–3.2)	2.2 (1.9–4.0)	1.5 (1.2–2.9)	0.051**
HbA1c (%)	5.0 ± 0.3	5.0 ± 0.3	5.0 ± 0.4	0.811*
Total cholesterol (mg/dL)	246.3 ± 38.8	243.4 ± 40.2	244.3 ± 48.1	0.974*
TGs (mg/dL)	177.0 (133.0–226.0)**a**	214.0 (192.8–300.0)**b**	187.0 (146.0–250.0)**a**	**0.007**** [c]
LDL (mg/dL)	134.5 ± 35.8	126.8 ± 30.8	134.7 ± 37.1	0.538*
HDL (mg/dL)	73.5 ± 15.1	68.8 ± 18.4	72.9 ± 13.5	0.352*
FSTL-1 (ng/mL)	241.3 (142.3–1005.8)	168.0 (124.4–314.9)	181.3 (160.2–466.4)	0.514**
(B) Comparison of metabolic parameters among the rs869247 genotypes in the GDM group				
Variables	CC (n = 24)	CT (n = 38)	TT (n = 10)	*p** value
FPG (mg/dL)	84.0 (78.8–92.0)	86.0 (77.0–94.5)	90 (78.5–96.0)	0.782**
Fasting Insulin (uIU/mL)	10.6 (6.7–13.1)	10.3 (6.9–16.7)	9.2 (6.3–13.8)	0.737**
HOMA-IR	2.1 (1.3–3.0)	1.7 (1.3–3.7)	1.6 (1.5–3.0)	0.489**
HbA1c (%)	5.2 ± 0.4	5.1 ± 0.4	5.0 ± 0.4	0.556*
Total cholesterol (mg/dL)	252.9 ± 44.1	246.3 ± 43.2	242.4 ± 22.2	0.763*
TGs (mg/dL)	195.0 (159.0–264.0)	231.5 (148.8–284.0)	173.0 (144.0–243.5)	0.547**
LDL (mg/dL)	136.6 ± 36.2	128.0 ± 36.5	134.2 ± 16.4	0.648*
HDL (mg/dL)	72.0 ± 17.3	72.0 ± 17.1	69.9 ± 8.2	0.937*
FSTL1 (ng/mL)	223.5 (164.7–406.5)	196.1 (132.3–486.9)	161.0 (138.0–231.7)	0.339**

*Mean ± standard deviation (SD); **Median (interquartile range, IQR) [The difference between median values with different lowercase letters in the same row was statistically significant (CC-CT: $p = 0.041$; CT-TT: $p = 0.018$)]. Bold values indicate statistical significance ($p < 0.05$). Different superscript letters (a, b) within the same row indicate statistically significant differences between the respective genotype groups based on post-hoc pairwise comparisons ($p < 0.05$).

[c]When maternal age, BMI, and HOMA-IR were included as covariates in a multivariable linear regression model using log-transformed TG levels, the association between the rs12173 variant and TGs was not statistically significant (adjusted $p = 0.386$, 95% CI: –0.038 to 0.098).

Pairwise comparisons between specific genotypes (e.g., CT vs. CC and CT vs. TT) were evaluated as post hoc exploratory analyses.

levels, and HOMA-IR values compared with the control group ($p < 0.05$).

Numerous studies have focused on FSTL1, particularly in relation to pathological processes in obesity and obesity-related metabolic diseases. Several recent studies have reported elevated circulating FSTL1 levels in obese individuals and diabetic rodents, with FSTL1 levels positively correlated with their BMI values [10]. Elevated serum FSTL1 levels have recently been associated with metabolic-associated fatty liver disease in obese children, emphasizing its widespread impact on metabolic dysregulation. In fact, recent studies have also proposed plasma FSTL1 as a reliable noninvasive diagnostic biomarker for advanced liver fibrosis, further emphasizing its potential clinical utility in systemic metabolic and inflammatory disorders [18]. This concept is further corroborated by comprehensive reviews describing the critical regulatory role of FSTL1 in various liver pathologies [19]. In contrast, FSTL1 levels were found to be lower in obese children than in the healthy control group in a recent study by Can et al. [20]. Based on these findings, the lack of difference in FSTL1 levels in our study may be attributable to the exclusion of obese patients, thereby minimizing the confounding effect of obesity when evaluating the role of FSTL1 in the development of GDM.

In a recent study examining the relationship between metabolic syndrome and FSTL1, patients with newly diagnosed metabolic syndrome had substantially higher serum FSTL1 levels than controls, and FSTL1 levels were positively correlated with FPG levels [9]. In the study conducted by Xu et al. [10], which investigated the relationship between FSTL1 and insulin resistance, FSTL1 protein expression was significantly upregulated in the adipose tissue of diabetic mice. Additionally, serum FSTL1 levels were found to be elevated in patients with type 2 diabetes mellitus and were correlated with lipid profiles, HOMA-IR, and glucose metabolism parameters [10]. On the other hand, the dynamic nature of this myokine is further highlighted by recent findings showing that moderate-intensity aerobic exercise significantly increases FSTL1 levels, which may prevent the development of atherosclerosis through various mechanisms, including improvement of endothelial function, suppression of smooth muscle cell proliferation, and reduction of arterial thickening [21]. Based on these findings, the role of FSTL1 in cardiometabolic diseases remains controversial, and further research is needed to fully understand the underlying mechanisms.

There are limited studies investigating the relationship between FSTL proteins and GDM in the literature. These studies focus on FSTL3, which is highly expressed in the placenta and belongs to the same family (TGFB family) as FSTL1. Increased glucose tolerance, insulin sensitivity, and pancreatic cell dysplasia were observed in FSTL3 knockout mice, resulting in higher insulin levels [22]. First-trimester FSTL3 levels were associated with GDM and glucose intolerance later in pregnancy [23]. It has also been reported that FSTL3 protein levels in the serum and placental tissues of women with GDM are significantly lower than those in controls [24]. These findings suggest that FSTL proteins may serve as biomarkers of glucose impairment in GDM. Despite these findings, the literature also includes studies that contradict these observations. In a study by Nanda et al. [25], first-trimester serum FSTL3 levels did not differ between women with GDM and healthy controls. Karageyim Karsidag et al. [26] reported no significant difference in serum FSTL3 levels between the GDM group and healthy controls. In a study investigating the relationship between circulating FST, activin A, and FSTL3 levels with glucose metabolism and fetal and neonatal adiposity, serum FST levels were significantly lower in women with GDM than in controls, whereas no differences were detected in serum activin A and FSTL3 levels [27]. To date, there are no published data on FSTL1 levels in patients with GDM, and our results show that serum FSTL1 concentrations are comparable between patients with GDM and healthy pregnant women.

There are limited published studies on gene polymorphisms within the FSTL protein family. In one study, circulating FSTL1 protein levels were evaluated in morbidly obese, super-obese, and non-obese individuals, and no significant association was found between FSTL1 levels and rs1057231 polymorphism genotypes in obese individuals [28]. In non-obese individuals with the GG genotype, serum FSTL1 levels were significantly higher compared to both GT and TT genotype carriers [10]. In women with PCOS, the FST gene was not identified as a susceptibility locus for PCOS. However, the rs3797297 SNP in the FST gene was reported to be associated with hyperandrogenemia in the disease [28]. In our study, we also found no statistically significant differences between patients with GDM and the control group in the distribution of the CC, CT, and TT genotypes and alleles. Furthermore, no association was observed between genotypes and FSTL1 levels in the GDM cohort.

Maternal metabolism becomes catabolic after the second trimester. Increased insulin resistance leads to increased levels of free fatty acids and serum TG levels, reaching their highest concentrations before birth. Free fatty acids and glycerol, which form TGs, can enter the fetal circulation and serve as energy sources for the fetus. In our initial unadjusted analysis, FSTL1 rs12173 CT genotype appeared to be significantly associated with hypertriglyceridemia. A positive correlation between circulating FSTL1 concentrations and elevated TG levels has also been documented in adult populations with metabolic syndrome. Moreover, it has recently been shown that FSTL1 levels increased significantly during endurance exercise and following recovery, correlating with lean body mass and lipolysis [9]. Although these findings suggest a possible link between FSTL1 and lipid metabolism, our multivariable

linear regression analysis revealed a different dynamic in our GDM cohort. After adjustment for maternal age, BMI, and HOMA-IR using log-transformed TG levels, the association between the CT genotype and hypertriglyceridemia lost its statistical significance. These findings indicate that the observed variation in lipid levels is primarily driven by baseline metabolic confounders, specifically adiposity and insulin resistance, rather than by a direct genetic effect.

Limitations

In our study, the small sample size represents a significant limitation. Additionally, the nonsignificant difference observed in FSTL1 levels between groups, despite the initial power calculation, suggests that the actual effect size in this specific population may be smaller than originally hypothesized. However, the selection of a specific subgroup of GDM patients with low risk for disease progression distinguishes our study from the previous studies in the literature. Because obesity and other high-risk factors known to elevate FSTL1 levels were excluded by design, our findings may not be generalizable to the broader, higher-risk GDM population.

5. Conclusions

To our knowledge, this is the first study to investigate FSTL1 levels and gene polymorphisms in GDM. The GDM group exhibited higher BMI, FPG, HbA1c, and HOMA-IR values than the control group. No statistically significant differences were observed between the GDM and control groups in the distribution of the CC, CT, and TT genotypes or allele frequencies of the FSTL1 rs12173 and rs869247 polymorphisms. There was no association between genotypes and FSTL1 levels in the GDM cohort. Although our initial unadjusted analysis suggested an association between the FSTL1 rs12173 CT genotype and hypertriglyceridemia, subsequent multivariable adjustments revealed that this relationship was primarily driven by maternal adiposity (BMI) and insulin resistance. Although FSTL1 is known to be involved in lipid mobilization [29], our exploratory findings indicate that the rs12173 variant is not an independent risk factor for dyslipidemia in pregnancies complicated by GDM. Furthermore, our findings indicate that circulating FSTL1 levels do not serve as a marker for GDM risk in this specific cohort. On the other hand, we selected a specific group of GDM patients at low risk for disease development to understand the role of FSTL1 without confounding factors such as obesity, age, and genetic predisposition. As such, our results may not be generalized to the broader GDM population. To elucidate the role of FSTL1 in metabolic disorders, further studies with a prospective design and large patient cohorts are warranted.

Availability of Data and Materials

The data and materials in the current study are available from the corresponding author upon reasonable request.

Author Contributions

EC: Investigation, resources, writing-original draft, visualization; GA: Conceptualization, resources, writing-original draft, visualization; TE: Investigation, conceptualization, writing-original draft and editing; EA: Conceptualization, resources, writing-original draft, visualization; FP: Investigation, resources, writing-original draft, visualization; NC: Conceptualization, resources, writing-review and editing, supervision, project administration. All authors contributed to editorial changes in the manuscript. 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.

Ethical Approval and Consent to Participate

The study protocol was approved by Mugla Sitki Kocman University Faculty of Medicine Medical Ethics Committee (Approval No: 13/II). Informed consent was obtained from all participants prior to their inclusion in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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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/CEOG50019.

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