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Serum Isthmin-1 in Gestational Diabetes Mellitus: A Case-Control Study Providing Preliminary Evidence of Elevated Levels and Association With Insulin Resistance

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: This study aimed to evaluate serum Isthmin-1 (ISM1) levels in pregnant women with and without gestational diabetes mellitus (GDM) and to assess its diagnostic value in comparison with established metabolic parameters.

Material/Methods: A case-control design was employed for this study, which enrolled 60 pregnant women between the ages of 20 and 40 years. Data collection took place over a 6-month period, from September 2023 through February 2024, at a tertiary-level hospital. Thirty women with GDM and 30 healthy pregnant controls were enrolled. Fasting and postprandial glucose, insulin, HOMA-IR, HbA1c, C-peptide, and ISM1 levels were measured. No multivariable adjustment was performed. ISM1 and C-peptide concentrations were analyzed using ELISA.

Results: When compared to the control group, serum ISM1 concentrations were notably elevated in women with GDM, with median values of 7.67 (6.7-10.8) ng/mL versus 6.96 (6.4-7.6) ng/mL, respectively, a difference that reached statistical significance ($P = 0.020$). ISM1 showed weak positive correlations with insulin ($r = 0.289$, 95% CI: 0.038-0.506, $P = 0.025$) and HOMA-IR ($r = 0.281$, 95% CI: 0.029-0.500, $P = 0.029$), although neither association survived correction for multiple comparisons. The diagnostic performance of ISM1 for GDM yielded an AUC of 0.674, with high specificity (93.3%) but low sensitivity (40%). Insulin and HOMA-IR demonstrated superior predictive values.

Conclusions: Serum ISM1 is modestly elevated in GDM and shows weak associations with insulin resistance parameters; its diagnostic performance is characterized by low sensitivity (40%) and an AUC of 0.674 (95% CI: 0.54-0.79) and is insufficient for independent clinical use. Any supportive role as part of a multi-marker panel requires confirmation in adequately powered, prospective studies.

Keywords: Biomarkers • Gestational Diabetes • Insulin Resistance

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Introduction

Gestational diabetes mellitus (GDM) refers to a state of impaired glucose tolerance that is identified for the first time during pregnancy [1] carrying significant health consequences for both the mother and the developing fetus. It typically develops in the second or third trimester and results from insufficient insulin response to the elevated insulin resistance induced by pregnancy, leading to hyperglycemia. The prevalence has been rising globally in recent years, in parallel with increasing rates of advanced maternal age, obesity, and sedentary lifestyles [2,3]. Hormones secreted by the placenta physiologically enhance insulin resistance and metabolism, as well as endogenous sex hormones [4]. In women with GDM, molecular mechanisms contributing to insulin resistance include proinflammatory cytokines and adipokine imbalances, which play a central role [5-7]. Although the metabolic basis of GDM is increasingly well understood, its clinical diagnosis still depends almost entirely on the oral glucose tolerance test, a procedure that requires fasting, repeated blood sampling over 2 hours, and is not always well tolerated, particularly in the third trimester. Against this background, there is genuine interest in identifying circulating biomarkers that could either support or refine GDM screening. Pregnancy is characterized by marked remodeling of adipose tissue, and the adipokines produced during this process are increasingly recognized as key regulators of placental function, maternal insulin sensitivity, and fetal metabolic programming. Biomarkers originating from this pathway are therefore good candidates for use in assessing gestational glucose intolerance.

Isthmin-1 (ISM1) is a recently characterized protein involved in inter-tissue communication, which is suggested to contribute to metabolic regulation. ISM1 is expressed in multiple tissues, including the skin, mucosal surfaces, and immune system cells. In line with this widespread expression pattern, recent studies have demonstrated that ISM1 participates in numerous biological processes, such as angiogenesis, organ homeostasis, immune responses, developmental signaling, and cancer [8-10]. The metabolic effects of ISM1 largely resemble insulin-like activity. It has been shown that ISM1 is secreted from adipocytes and enhances cellular glucose uptake via an insulin-independent mechanism. ISM1 activates the PI3K/Akt signaling pathway independently of insulin and insulin-like growth factor receptors, thereby promoting glucose transport in adipose tissue and improving peripheral insulin sensitivity [11-13]. Observational studies conducted in various populations have suggested that circulating ISM1 levels are associated with obesity and diabetes [14]. In a recent case-control study, serum ISM1 levels were found to be markedly elevated in pregnant women with a diagnosis of GDM relative to their healthy counterparts [15]. Although current evidence indicates a possible link between ISM1 and glucose metabolism during pregnancy, the causal direction of this relationship and the underlying mechanisms remain unclear.

Research on ISM1 in the context of human pregnancy is still in its early stages, and its potential relevance to GDM remains poorly understood. Given the limited and somewhat inconsistent evidence available, we conducted this study to compare serum ISM1 levels between women diagnosed with GDM and normoglycemic pregnant controls, to examine how ISM1 relates to markers of insulin resistance, and to determine whether it offers any meaningful diagnostic information in this setting.

Material and Methods

Setting and Design

This case-control study was conducted at the Department of Obstetrics and Gynecology of Ordu University Training and Research Hospital between September 2023 and February 2024. A total of 60 pregnant women aged between 20 and 40 years, all with singleton pregnancies and who completed antenatal follow-up and delivery at the same institution, were included. Participants were enrolled consecutively from the obstetrics outpatient clinic during routine antenatal visits. Controls were selected from women who attended the same clinic for their 75 g OGTT at the same gestational period and who met the eligibility criteria. No formal matching was performed; however, the groups were compared for key demographic variables, and no significant differences were identified in age, body mass index, height, or weight.

The study was approved by the Clinical Research Ethics Committee of Ordu University (date: September 15, 2023, approval no: #2023/231). The study was conducted in full compliance with the ethical standards set forth by the Declaration of Helsinki, and all participants provided their written informed consent prior to enrollment. The study population was drawn exclusively from pregnant women attending the obstetrics outpatient clinic for routine OGTT screening. No participants from other concurrent studies conducted at the same institution were enrolled in this cohort, and no there was no participant overlap with previously published work from this laboratory.

Thirty participants were diagnosed with GDM, while the remaining 30 had normal results in the oral glucose tolerance test (OGTT) and were considered healthy controls. The diagnosis of GDM was based on the results of a 75 g OGTT performed at 24 to 28 weeks of gestation. Diagnostic criteria were based on the thresholds recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG), requiring at least 1 of the following values to be exceeded: fasting ≥ 92 mg/dL, 1-hour ≥ 180 mg/dL, or 2-hour ≥ 153 mg/dL.

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We excluded women with multiple gestations, pregestational diabetes, chronic diseases (including hypertension, thyroid disorders, and autoimmune diseases), preeclampsia, intrahepatic cholestasis, a history of preterm birth, conception via assisted reproductive techniques, or active smoking.

Blood Sampling and Serum Preparation

Fasting venous blood samples (approximately 8 mL) were obtained from the antecubital vein of all participants in the morning, following at least 8 hours of overnight fasting, on the same day as the OGTT. Samples were transferred into 2 biochemistry tubes without anticoagulant and centrifuged at 3000 rpm for 10 minutes. A portion of the resulting serum was used for immediate laboratory analysis, while the remaining part was stored at -80 °C until the measurement of ISM1 and C-peptide levels. Serum samples were collected concurrently with the fasting glucose measurement of the OGTT.

Laboratory Analyses

Biochemical parameters were analyzed in the biochemistry laboratory of Ordu University. Fasting and postprandial glucose levels, OGTT values (0, 60, and 120 minutes), insulin, homeostasis model assessment-estimated insulin resistance (HOMA-IR), hemoglobin A1c (HbA1c), urea, creatinine, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels were measured using the Cobas 6000 c 501 and e 601 analyzers (Roche Diagnostics, Mannheim, Germany). Complete blood count parameters, including hemoglobin, hematocrit, platelet, and leukocyte levels, were assessed using the Sysmex XN-1000 (Sysmex Corporation, Kobe, Japan). Coagulation parameters, including activated partial thromboplastin time (APTT) and international normalized ratio (INR), were measured using the Cobas t 511 analyzer (Roche Diagnostics). HbA1c levels were analyzed using the high-performance liquid chromatography (HPLC) method with the Arkray Adams HA-8180T system (Japan).

Measurement of Isthmin-1 and C-Peptide

ISM1 and C-peptide levels were measured using the ELISA method from stored serum samples. ISM1 concentrations were measured using a commercially available sandwich ELISA kit (Human ISM1 ELISA Kit; BT Lab, Jiaxing Korain Biotech, Zhejiang, China; Cat. No. E7643Hu, Lot No: E7643Hu). The assay detection range was 0.38 to 24 ng/mL, with a reported analytical sensitivity of 0.17 ng/mL. The manufacturer-specified intra-assay and inter-assay coefficients of variation were <8% and <10%, respectively. All measured ISM1 concentrations fell within the validated analytical range of the kit, and no sample dilution was required. Serum samples were stored at -80 °C and subjected to only a single freeze–thaw cycle immediately prior to ELISA analysis. GDM and control samples were analyzed on the same ELISA plates, with the 2 groups balanced across plates, and all measurements were performed using kits from a single lot. The assay was based on a sandwich ELISA principle, with optical density measured at 450 nm. C-peptide levels were measured using a C-Peptide ELISA Kit manufactured by Elabscience (Texas, USA; Lot No: E-EL-H0121). All ELISA procedures were performed by the same technician in accordance with the manufacturer's protocol.

Statistical Analysis

Sample size was calculated using G*Power software (v3.1.2). Considering a Cohen's effect size coefficient of $d = 0.80$, a minimum of 30 participants per group was required to achieve 80% statistical power. This effect size was selected based on the magnitude of ISM1 differences reported in the only available comparable study at the time of study planning [16]. It should be acknowledged, however, that the large effect size assumption ($d = 0.80$) may not adequately reflect the modest group differences and weak correlations ultimately observed in the present study. Post hoc power estimates for the correlation analyses were as follows: to detect a correlation of $r = 0.28$ with 80% power at $\alpha = 0.05$ (2-tailed), approximately 130 participants would be required; for an AUC of 0.674, the 95% CI (0.54-0.79) indicates substantial imprecision, consistent with insufficient power for a tightly bounded diagnostic estimate.

Data were analyzed using MedCalc statistical software (version 20.009; Ostend, Belgium). The Shapiro-Wilk test was used to assess normality. Descriptive statistics were presented as mean ± standard deviation, median (25th-75th percentile), frequencies, and percentages. For comparisons between 2 independent groups, the independent samples t test was used for parametric variables, and the Mann-Whitney U test was used for nonparametric variables. Correlations between numerical variables were analyzed using Pearson or Spearman correlation tests depending on the distribution characteristics. Given the exploratory nature of the correlation analyses and the relatively small number of primary hypotheses, a formal correction for multiple comparisons (eg, Bonferroni) was not applied. Accordingly, correlations involving ISM1 should be interpreted with caution, and significant associations other than those with insulin and HOMA-IR are considered hypothesis-generating.

To assess the discriminative performance of ISM1 in diagnosing GDM, a receiver operating characteristic (ROC) analysis was conducted, and the area under the curve (AUC), cut-off value, sensitivity, specificity, and positive and negative predictive values were calculated. A binary logistic regression model with GDM as the outcome and ISM1, age, and BMI as covariates was fitted to assess the independence of the ISM1 association. Additionally, a partial correlation between ISM1 and

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Table 1. Demographic characteristics of the GDM and control groups.

	Groups				*P*
	Control (n = 30)		GDM (n = 30)		
Age (years)	28.7	4.5	31.1	4.9	0.054
Gestational age at birth (weeks)	39.5	(39-40)	38	(38-39)	< 0.001**
Gravida	1	(1-2)	2	(1-3)	0.039**
Parity	0	(0-1)	1	(0-2)	0.064
Live births	0	(0-1)	1	(0-2)	0.094
Type of birth					
CS	16	53.3%	22	73.3%	0.111
NSB	14	46.7%	8	26.7%	
Weight (kg)	80	(75-85)	80	(72-89)	0.733
Height (cm)	160.5	(158-164)	161	(160-165)	0.501
BMI (kg/m^2)	30.5	(28-33)	30	(28-35)	0.947
Fetal sex					
Female	18	60.0%	14	46.7%	0.305
Male	12	40.0%	16	53.3%	
Birth weight (gr)	3413	378	3411	586	0.989
Intensive care requirement					
No	28	93.3%	24	80.0%	0.132
Yes	2	6.7%	6	20.0%	
Apgar score (1-minute)	9	(8-9)	9	(9-9)	0.218
Apgar score (5-minute)	10	(9-10)	10	(9-10)	0.224

* Significant difference at < 0.05 level according to independent *t* test, Means and Standard deviations (SD) are presented.
** Significant difference at < 0.05 level according to Mann-Whitney U test, Medians are presented and 25p-75p are shown in parentheses. *** Significant difference at < 0.05 level according to chi-square test, N [%] presented. CS, Cesarean section; NSB, normal spontaneous birth.

HOMA-IR was computed with BMI as the covariate. A *P* value of <0.05 was considered statistically significant for all analyses.

Results

A total of 60 pregnant women were included in the study: 30 with GDM and 30 healthy controls. There were no significant differences between the groups in terms of age, body mass index, height, or weight ($P > 0.05$). The gestational age at delivery was significantly lower in the GDM group compared to the control group (38 [38-39] weeks vs 39.5 [39-40] weeks; $P < 0.001$). Gravida count was significantly higher among women with GDM (2 [1-3] vs 1 [1-2]; $P = 0.039$). This difference in gestational age at delivery between the groups may reflect obstetric management decisions related to GDM rather than representing a primary confounder for the biochemical findings reported. Since all ISM1 measurements were obtained at 24 to 28 weeks of gestation (prior to delivery), the difference in delivery gestational age is unlikely to have introduced systematic bias in the biomarker assessments. No significant differences were observed between the groups regarding parity, number of live births, mode of delivery, or neonatal sex ($P > 0.05$). Neonatal birth weight and Apgar scores at 1 and 5 minutes were similar. Although the need for neonatal intensive care was more common in the GDM group, the difference was not significant ($P = 0.132$) (**Table 1**).

Laboratory tests revealed serum ISM1 levels were significantly higher in the GDM group than in the control group (7.67

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Table 2. Comparison of GDM and control groups in terms of laboratory findings.

	Groups				*P*
	Control (n = 30)		GDM (n = 30)		
Isthmin-1 (ng/mL)	6.96	(6.4-7.6)	7.67	(6.7-10.8)	0.020**
C-Peptide (ng/mL)	0.92	(0.6-1.4)	1.25	(0.8-1.8)	0.051
FBG (mg/dL)	80	(77-85)	87	(82-90)	0.003**
PPBG (mg/dL)	102	(98-114)	129	(112-146)	<0.001**
Insulin (µU/mL)	7.5	2.6	22	11.0	<0.001*
Homa-IR (Index)	1.5	0.5	5.2	2.5	<0.001*
HbA1c (%)	5.3	(5.1-5.4)	5.4	(5.1-6)	0.136
Glucose tolerance test (fasting)	89	(79-90)	95	(92-100)	<0.001**
Glucose tolerance test (1-hour)	144	(135-150)	187	(161-195)	<0.001**
Glucose tolerance test (2-hour)	122	(103-125)	134	(114-166)	0.009**
Urea (mg/dL)	7	(5-13)	6	(5-7)	0.051
Creatinine (mg/dL)	0.47	(0.4-0.5)	0.48	(0.45-0.5)	0.732
Total protein (g/L)	63	6.5	63	5.0	0.982
Albumin (g/L)	35	(33-40)	34	(32-39)	0.233
ALT (IU/L)	11	(9-15)	9	(8-12)	0.229
AST (IU/L)	15	(12-19)	15	(12-17)	0.917
LDH (IU/L)	179	(173-197)	190	(163-201)	0.842
Hb (g/dL)	11	1.1	12	1.1	0.240
Hct (%)	34	2.8	35	3.0	0.357
PLT (10^3/L)	211	(185-256)	205	(191-247)	0.663
WBC (10^3/L)	9	(8.5-10.8)	9	(7.5-10.5)	0.534
APTT (second)	27	2.9	26	2.7	0.165
INR	0.89	(0.8-0.9)	0.9	(0.86-0.9)	0.729

* Significant difference at < 0.05 level according independent *t* test. Means and standard deviations (SD) are presented.
** Significant difference at < 0.05 level according to Mann-Whitney U test. Medians are presented and 25p-75p are shown in parentheses. ALT, alanine aminotransferase, APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BMI, body mass index; C-Peptide, connecting peptide; FBG, fasting blood glucose; GDM, gestational diabetes mellitus; Hb, hemoglobin; HbA1c, glycated hemoglobin; Hct, hematocrit; HOMA-IR, homeostasis model assessment of insulin resistance, INR, international normalized ratio; LDH, lactate dehydrogenase; PLT, platelet count; PPBG, postprandial blood glucose; WBC, white blood cell count.

[6.7-10.8] ng/mL vs 6.96 [6.4-7.6] ng/mL; $P = 0.020$). As anticipated, fasting glucose (87 [82-90] vs 80 [77-85] mg/dL; $P = 0.003$), postprandial glucose (129 [112-146] vs 102 [98-114] mg/dL; $P < 0.001$), insulin (22 ± 11.0 vs 7.5 ± 2.6 µU/mL; $P < 0.001$), and HOMA-IR (5.2 ± 2.5 vs 1.5 ± 0.5; $P < 0.001$) levels were significantly higher in the GDM group. HbA1c was similar in the 2 groups ($P = 0.136$). In OGTT results, fasting, 1-hour, and 2-hour glucose levels were all significantly higher in the GDM group compared to controls ($P < 0.001$, $P < 0.001$, and $P = 0.009$, respectively). Other biochemical, hematologic, and coagulation parameters were similar between the groups ($P > 0.05$) (**Table 2**).

According to the correlation analysis, serum ISM1 levels showed a significant positive correlation with insulin (r = 0.289, $P = 0.025$) and HOMA-IR (r = 0.281, $P = 0.029$). No significant

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Table 3. Correlation between serum Isthmin-1 concentrations and other laboratory parameters.

			Isthmin-1 (ng/mL)	
			N = 60	
C-Peptide (ng/mL)	r	(95% CI)	0.049	(-0.207-0.299)
	p		0.709	
FBG (mg/dL)	r	(95% CI)	0.081	(-0.177-0.328)
	p		0.539	
PPBG (mg/dL)	r	(95% CI)	0.113	(-0.145-0.357)
	p		0.391	
Insulin (µU/mL)	r	(95% CI)	0.289	(0.038-0.506)
	p		0.025*	
Homa-IR (Index)	r	(95% CI)	0.281	(0.029-0.500)
	p		0.029*	
HbA1c (%)	r	(95% CI)	-0.116	(-0.359-0.143)
	p		0.379	
Glucose tolerance test (fasting)	r	(95% CI)	0.070	(-0.187-0.318)
	p		0.596	
Glucose tolerance test (1-hour)	r	(95% CI)	0.227	(-0.029-0.455)
	p		0.081	
Glucose tolerance test (2-hour)	r	(95% CI)	0.244	(-0.010-0.469)
	p		0.060	
Urea (mg/dL)	r	(95% CI)	-0.018	(-0.271-0.237)
	p		0.890	
Creatinine (mg/dL)	r	(95% CI)	0.000	(-0.254-0.254)
	p		1.000	
Total protein (g/L)	r	(95% CI)	0.000	(-0.254-0.254)
	p		1.000	
Albumin (g/L)	r	(95% CI)	-0.033	(-0.284-0.223)
	p		0.805	
ALT (IU/L)	r	(95% CI)	0.068	(-0.189-0.316)
	p		0.606	
AST (IU/L)	r	(95% CI)	0.159	(-0.099-0.396)
	p		0.226	
LDH (IU/L)	r	(95% CI)	0.172	(-0.085-0.408)
	p		0.188	
Hb (g/dL)	r	(95% CI)	0.111	(-0.147-0.355)
	p		0.397	
Hct (%)	r	(95% CI)	0.199	(-0.058-0.431)
	p		0.128	

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Table 3 continued. Correlation between serum Isthmin-1 concentrations and other laboratory parameters.

			Isthmin-1 (ng/mL)	
			N = 60	
PLT (10^9/L)	r	(95% CI)	0.085	(-0.173-0.331)
	p		0.521	
WBC (10^9/L)	r	(95% CI)	-0.107	(-0.351-0.151)
	p		0.416	
APTT (seconds)	r	(95% CI)	0.014	(-0.241-0.267)
	p		0.919	
INR	r	(95% CI)	-0.050	(-0.300-0.207)
	p		0.705	

* Significant difference at <0.05 level according Spearman correlation. correlation coefficient (r) presented. With 22 simultaneous correlation tests. the Bonferroni-corrected threshold for significance is $P < 0.0023$; the Benjamini–Hochberg FDR threshold ($q < 0.05$) yields a similar cut-off. Neither ISM1–insulin nor ISM1–HOMA-IR meets these corrected thresholds (uncorrected $P = 0.025$ and $P = 0.029$. respectively). All correlations should therefore be interpreted as exploratory and hypothesis-generating.

Table 4. Results of binary logistic regression analysis (dependent variable: GDM).

Variable	OR	95% CI	*p*
Isthmin-1 (ng/mL)	1.60	1.07-2.39	0.023
Age (years)	1.13	0.98-1.32	0.095
BMI (kg/m^2)	1.02	0.92-1.13	0.686

OR, odds ratio; CI, confidence interval. N = 60.

correlations were observed between ISM1 levels and other metabolic or hematologic parameters ($P > 0.05$) (**Table 3**).

To explore whether the association between ISM1 and GDM was independent of potential confounders, a binary logistic regression model was fitted with GDM status as the outcome and serum ISM1, age, and BMI as covariates. In this model, serum ISM1 remained a statistically significant predictor of GDM (OR = 1.60, 95% CI: 1.07-2.39; $P = 0.023$), while age (OR = 1.13, 95% CI: 0.98-1.32; $P = 0.095$) and BMI (OR = 1.02, 95% CI: 0.92-1.13; $P = 0.686$) did not reach statistical significance (**Table 4**). Additionally, the positive correlation between ISM1 and HOMA-IR remained statistically significant after adjustment for BMI using partial correlation analysis (r = 0.32, $P = 0.012$).

Based on ROC analysis, the area under the curve (AUC) value for serum ISM1 in predicting GDM was 0.674 (95% CI = 0.54-0.79, $P = 0.013$). At a cut-off value of >8.87 ng/mL, ISM1 showed a sensitivity of 40%, specificity of 93.3%, positive predictive value of 85.7%, and negative predictive value of 60.9. The AUC values of other metabolic parameters were calculated as follows: HOMA-IR: 0.976, insulin: 0.941, fasting glucose: 0.720, and C-peptide: 0.647 (all $P < 0.05$) (**Table 5, Figure 1**). Among the independent comparators—C-peptide (AUC 0.647) and HbA1c (AUC 0.611)—ISM1 (AUC 0.674) performed comparably, and its 93.3% specificity notably exceeded that of both. HOMA-IR (AUC 0.976) and insulin (AUC 0.941) demonstrated substantially higher discriminatory capacity; however, as both incorporate fasting glucose—a constituent variable of the IADPSG diagnostic threshold—this comparison involves a degree of analytical circularity and should be interpreted with appropriate caution.

Discussion

In this study, serum ISM1 levels were investigated in the context of GDM, with a particular focus on their relationship with glucose metabolism. The results revealed that ISM1 levels were significantly altered in GDM and were positively associated with markers of insulin resistance, suggesting a role for ISM1 as a biomarker in the metabolic alterations observed during gestational diabetes.

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Table 5. Diagnostic values of serum Isthmin-1 concentrations and laboratory parameters associated with GDM.

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC (95% CI)		*P*
FBG (mg/dL) 'reference marker'	>81	76.7	63.3	67.6	73.1	0.720	(0.590-0.850)	<0.001*
Insulin (µU/mL) 'reference marker'	>10	86.7	93.3	92.9	87.5	0.941	(0.882-0.999)	<0.001*
Homa-IR (Index) 'reference marker'	>2.4	86.7	96.7	96.3	87.9	0.976	(0.947-1.000)	<0.001*
Isthmin-1 (ng/mL)	>8.87	40.0	93.3	85.7	60.9	0.674	(0.537-0.812)	0.013*
C-Peptide (ng/mL)	>0.99	63.3	66.7	65.5	64.5	0.647	(0.506-0.789)	0.041*
HbA1c (%)	>5.8	26.7	96.7	88.9	56.9	0.611	(0.467-0.755)	0.130

Reference markers: FBG. insulin. and HOMA-IR incorporate fasting glucose as a constituent variable—the same parameter used in the IADPSG diagnostic threshold for GDM. Their high diagnostic performance therefore partly reflects definitional circularity rather than fully independent predictive capacity. AUC, area under the curve; C-Peptide, connecting peptide; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; NPV, negative predictive value; PPV, positive predictive value.

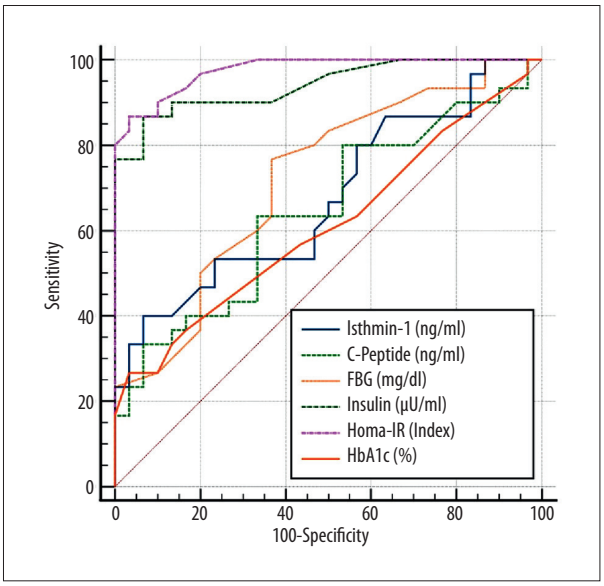

Figure 1. ROC curves demonstrating the diagnostic performance of serum Isthmin-1, C-peptide, fasting blood glucose (FBG), insulin, HOMA-IR, and HbA1c in distinguishing GDM cases from healthy pregnant controls. AUC values with 95% confidence intervals: Isthmin-1, 0.674 (95% CI: 0.537-0.812); C-peptide, 0.647 (95% CI: 0.506-0.789); FBG, 0.720 (95% CI: 0.590-0.850); insulin, 0.941 (95% CI: 0.882-0.999); HOMA-IR, 0.976 (95% CI: 0.947-1.000); HbA1c, 0.611 (95% CI: 0.467-0.755). HOMA-IR, insulin, and FBG are labeled as reference markers (see Table 5 footnote).

Data on the effects of the Isthmin protein family on pregnancy physiology are quite limited in the current literature. We found serum ISM1 levels were significantly higher in pregnant women with GDM compared to healthy controls. Although the diagnostic performance of ISM1 was modest—reflected by an AUC of 0.674 with low sensitivity (40%)—it achieved high specificity (93.3%), suggesting it may have a role as a confirmatory or supplementary marker rather than a primary screening tool. This finding appears to be consistent with the limited knowledge on this subject. The principal prior study in this area, by Şentürk et al, similarly reported significantly elevated serum ISM1 in pregnant women with GDM compared to healthy controls at the same gestational window (24-28 weeks). However, a direct numerical comparison is not meaningful; Şentürk et al used a different ELISA platform (Wuhan Fine Biotech; detection range 15.625-1000 pg/mL) and reported values in pg/mL (medians approximately 2785-3244 pg/mL), whereas the present study used the BT Lab kit (detection range 0.38-24 ng/mL) and reports values in ng/mL (medians 6.96-7.67 ng/mL) — a discrepancy of approximately 3 orders of magnitude, attributable to assay-specific quantification rather than genuine biological difference. Both studies confirm the directional association between ISM1 and GDM, but their numerical results cannot be directly compared. The diagnostic profile also differed between the 2 studies. Şentürk et al reported balanced sensitivity and specificity (62.5%/62.5%), whereas the present study yielded a high-specificity/low-sensitivity profile (93.3%/40%), which may reflect differences in sample composition, gestational characteristics, or assay-platform effects. This disagreement shows the need for cross-platform standardization in future ISM1 research.

ISM1 was initially shown to promote glucose uptake via insulin-independent pathways, and it has been proposed that its levels can increase as a compensatory response to hyperglycemia [15]. In contrast, Martinez et al reported that levels

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of Isthmin-2 (ISM2) were significantly lower in cases of preeclampsia, showing no associations between ISM1 levels and clinical findings such as preeclampsia and gestational hypertension based on serum concentrations and placental tissue expression. They suggested that ISM2 plays an important role in the pathophysiology of preeclampsia, particularly in relation to impaired angiogenesis [16]. The increased ISM1 levels observed in our study in the context of GDM may indicate that these proteins are involved in physiological stress responses during pregnancy. The fact that members of the Isthmin family are variably affected by pregnancy complications such as GDM and preeclampsia suggests that their expression is regulated in a tissue- or condition-specific manner. From this perspective, examining ISM1 levels in various pregnancy-related complications may be of clinical interest. In GDM, the elevation in this adipokine raises the question of whether the increase is a secondary response or a direct component of the underlying pathophysiology. However, since our study was not designed to investigate the mechanistic pathways of this relationship, no definitive conclusions can be drawn on this matter. It should also be noted that serum ISM1 does not permit identification of its tissue of origin during pregnancy. Whether the elevated circulating ISM1 in GDM originates primarily from adipose tissue, placenta, or other sources cannot be determined from serum measurements alone, and tissue-level studies are needed.

ISM1 is one of several adipokines that have been reported to differ between women with and without GDM. Leptin and chemerin tend to be elevated in GDM, while adiponectin and meteorin-like protein (Metrnl) are generally reduced. Asprosin, a more recently characterized adipokine, has also been investigated in this context. Unlike many of these adipokines, ISM1 is proposed to exert insulin-mimetic effects and to promote glucose uptake independently of insulin signaling, which may account for its apparently compensatory elevation in GDM. Whether ISM1 adds independent information to these established adipokines or whether its levels are collinear with them is an important question for future multi-adipokine studies.

Our study was conducted exclusively in a pregnant population. Previous studies in non-pregnant individuals with type 2 diabetes mellitus (T2DM) have suggested that ISM1 levels may be associated not only with the presence of diabetes but also with metabolic complications. Liao et al conducted a cross-sectional study in which serum ISM1 concentrations were found to be substantially elevated in adults diagnosed with T2DM relative to non-diabetic controls; however, this elevation did not appear to be related to the development of diabetic peripheral neuropathy. Notably, the same investigation revealed that obese women exhibited significantly reduced ISM1 levels [17]. Several studies investigating the relationship between ISM1 and renal function in individuals with T2DM have reported noteworthy findings. ISM1 levels showed an inverse relationship with eGFR, while a positive and independent association was found between ISM1 and albuminuria [18,19]. Patients with micro- and macro-albuminuria had significantly higher ISM1 levels; however, no significant correlations were found between ISM1 and markers of insulin resistance such as HOMA-IR and C-peptide [19]. Additionally, a study by Deng et al in individuals with primary hypertension revealed that ISM1 was associated with both urinary sodium excretion and insulin resistance [20]. Regarding lipid metabolism, some studies have demonstrated an inverse relationship between ISM1 levels and high-density lipoprotein cholesterol in patients with T2DM. ISM1 levels were found to be elevated particularly in individuals with low high-density lipoprotein cholesterol, suggesting that ISM1 may act as a marker of metabolic stress [21]. In a study by Wang et al, ISM1 levels were significantly higher in individuals with T2DM who had macrovascular complications, and ISM1 was positively correlated with hemoglobin A1c, HOMA-IR, triglycerides, and fasting glucose levels [22]. Recent studies have also highlighted a potential link between ISM1 and non-alcoholic fatty liver disease (NAFLD) beyond glucose metabolism. In individuals with metabolic syndrome and T2DM, serum ISM1 levels were found to reflect NAFLD status and were reported to improve glucose tolerance and insulin sensitivity [23]. Furthermore, in T2DM patients, a significant increase in serum ISM1 levels was observed after 12 weeks of metformin treatment, and this increase was inversely related to reductions in low-density lipoprotein cholesterol. The authors suggested ISM1 could serve as a useful biomarker for tracking how patients respond to treatment [24]. Notably, when age and BMI were included as covariates in a logistic regression model, serum ISM1 retained its independent association with GDM (OR = 1.60, 95% CI: 1.07-2.39), and its correlation with HOMA-IR remained significant after partial adjustment for BMI ($r = 0.32$, $P = 0.012$), suggesting that these associations are not due solely to shared adiposity-related variance.

However, some studies have reported an inverse relationship between ISM1 levels and T2DM and insulin resistance. Alshawaf et al examined obese and non-obese individuals and found that serum ISM1 concentrations were considerably lower among those with obesity; furthermore, this reduction showed a negative correlation with both insulin resistance and NAFLD [25]. Another study further proposed that elevated ISM1 levels might be linked to a lower likelihood of developing T2DM. Collectively, these inconsistent findings suggest that the strength and direction of ISM1's associations can vary [26]. Collectively, these inconsistent findings suggest that the strength and direction of ISM1's associations may vary with distinct clinical features in the context of T2DM. However, the direction of the findings is sometimes contradictory. This inconsistency may be attributed to methodological differences

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among studies, variations in study populations, inclusion and exclusion criteria, the sensitivity of the ELISA kits used, and the timing of sample collection. The increase in ISM1 observed in pregnant women with GDM in our study may represent the pregnancy-specific counterpart of the metabolic stress response seen in T2DM. Nevertheless, given the differing physiological conditions, the specific mechanisms underlying ISM1 elevation during pregnancy warrant further investigation.

When interpreting the comparative diagnostic performance of these biomarkers, an important caveat deserves mention. Both insulin and HOMA-IR incorporate fasting blood glucose as a constituent variable—the same parameter that forms one of the IADPSG diagnostic thresholds for GDM. Their high AUC values therefore partly reflect this definitional overlap rather than truly independent predictive capacity, and should be interpreted accordingly. ISM1, by contrast, is structurally unrelated to current diagnostic criteria, making its modest but statistically significant discriminative performance arguably more informative from a biomarker discovery standpoint. However, its high specificity coupled with low sensitivity suggests that ISM1 is unlikely to function as a standalone screening tool. A more practical avenue may be its incorporation into a multi-marker panel, where it could complement higher-sensitivity markers such as fasting glucose in a sequential diagnostic algorithm. Whether ISM1 adds meaningful incremental value in this setting, particularly among women at borderline risk on conventional testing, is a question worth pursuing in future studies.

In our study, ISM1 levels were evaluated at a single time point, and no conclusions could be drawn regarding the pathophysiological processes underlying ISM1 and GDM development. Preclinical studies have proposed that ISM1 may promote glucose uptake via insulin-independent pathways, including GLUT4 translocation through PI3K-AKT-mTORC2 signaling, but these mechanisms were not measured in the present study [10,13,27]. It has also been reported that ISM1 can exert anti-inflammatory effects by inhibiting the release of proinflammatory cytokines via NF-κB suppression [11]. Animal studies have further suggested potential roles for ISM1 in suppressing hepatic glucose production and lipogenesis, but the clinical relevance of these findings in pregnant women remains speculative [13,19,28]. The elevation in ISM1 observed in the GDM group in our study suggests that pregnancy-specific metabolic alterations can influence this adipokine. However, these mechanistic inferences are drawn from preclinical or non-pregnant population studies, and our cross-sectional design does not permit causal interpretation of the ISM1 elevation observed in GDM. However, further advanced and mechanistic studies are needed to clarify this relationship at the pathophysiological level.

The clinical implications of GDM extend well beyond glucose metabolism, affecting a broad range of maternal and neonatal outcomes. A retrospective single-center study by Kalandyk-Osinko et al demonstrated in that women with GDM have significantly longer hospital stays, higher rates of cesarean delivery, and greater gestational weight gain within recommended ranges compared to healthy controls, findings that underscore the systemic burden this condition places on both mother and child. Beyond these obstetric outcomes, the inflammatory dimension of GDM has attracted increasing attention. Gorczyca et al recently reported significantly lower postpartum IL-37 concentrations between women with a prior history of GDM and those without any such diagnosis, suggesting that anti-inflammatory dysregulation can persist beyond delivery and could serve as an early marker of long-term metabolic risk. Viewed alongside these findings, the elevation in serum ISM1 observed in our GDM group may be a component of a broader, multifaceted metabolic and immunological stress response characteristic of this condition. Whether ISM1 interacts with inflammatory mediators such as IL-37 in the context of GDM-associated insulin resistance needs further investigation.

Limitations

This study has several limitations. Most fundamentally, the sample was small and drawn from a single center, which constrains both statistical power and the extent to which results can be generalized. Because participants were not formally matched and key variables were not collected, residual confounding from unmeasured factors cannot be excluded. These include dietary habits, physical activity levels, socioeconomic status, ethnicity, prior history of GDM, family history of type 2 diabetes mellitus, and gestational weight gain, all of which may independently influence insulin resistance and adipokine profiles during pregnancy. Serum ISM1 was measured only once, at the time of the OGTT, and we therefore have no information about how levels change across gestation or whether early ISM1 alterations might anticipate the development of glucose intolerance. The manufacturer's manual did not provide data on potential cross-reactivity with ISM2—a structurally-related protein sharing the TSR1/AMOP domain architecture with ISM1—nor on spike-recovery or dilution linearity. These parameters were therefore not independently verified in our laboratory, which is a recognized limitation for a biomarker-focused study of this kind. The lack of multivariable adjustment is a further constraint, as is the absence of multiple testing correction in the correlation analyses, and associations beyond those with insulin and HOMA-IR should be interpreted cautiously. Neonatal outcomes were not analyzed in relation to serum ISM1 levels, which represents an avenue for future investigation. The statistically significant difference in gestational age at delivery (38 vs 39.5 weeks, $P < 0.001$) is a group imbalance. As ISM1 measurements were obtained at 24 to 28 weeks—prior to delivery—this difference is unlikely to have directly confounded the biomarker results; however, it may reflect underlying disease severity or differential obstetric

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management in the GDM group. A further limitation concerns assay comparability. The ISM1 concentrations reported here are approximately 3 orders of magnitude higher than those reported by Şentürk et al using a different ELISA kit, precluding direct numerical comparison across studies. Cross-validation of ISM1 quantification on a second analytical platform or the use of certified reference materials would be necessary to establish assay-to-assay agreement and to support inter-study comparisons. Lastly, HOMA-IR, while widely used as a proxy for insulin resistance, was originally validated in non-pregnant adults and has recognized limitations in the context of mid-to-late pregnancy, where the physiological insulin resistance of the third trimester can distort its interpretation. Its use as the primary correlational comparator for ISM1 in this study should be understood with this caveat in mind.

Conclusions

Serum ISM1 levels were significantly higher in pregnant women with GDM than in healthy controls, and showed modest but statistically significant correlations with insulin and HOMA-IR. At its optimal diagnostic threshold, ISM1 achieved high specificity but low sensitivity, and its overall discriminative performance fell well short of established markers such as insulin and HOMA-IR, a comparison that is itself complicated by the partial definitional overlap between these markers and the GDM diagnostic criteria. Taken together, the data do not support ISM1 as a standalone diagnostic tool, but leave open the possibility that it may contribute useful information as part of a broader biomarker panel. These findings are best regarded as preliminary. Larger, prospective studies incorporating longitudinal ISM1 measurements and appropriate confounder adjustment will be necessary to determine whether ISM1 holds a meaningful place in the clinical evaluation of GDM. Given its high specificity (93.3%) in the context of low sensitivity, a more realistic near-term clinical application may be as a rule-in test applied to women with borderline OGTT results, rather than as a general screening tool.

Institutions Where Work Was Done

This study was conducted at the Obstetrics and Gynecology Clinic of Ordu University Training and Research Hospital and the Obstetrics and Gynecology Clinic of Ordu Private Sevgi Hospital, Ordu, Türkiye.

Institutional Review Board Statement

The research was approved by the Clinical Research Ethics Committee (date: September 15, 2023; approval no: #2023/231). The official approval document is available upon request.

Informed Consent Statement

Informed consent was obtained from all participants, as clearly stated in the main body of the manuscript.

Data Availability Statement

The data used in this study are available upon reasonable request.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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