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ORIGINAL ARTICLE

Evaluation of the Potential Clinical Utility of Maternal Serum Biomarkers for Risk Assessment in Gestational Diabetes

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SUMMARY

Background: **The exact relationships of circulating fibronectin, SHBG, and ILGF-1 with T2DM and GDM remain inconsistent. Therefore, in this study we evaluate their associations in T2DM and GDM. Additionally, we evaluate their correlations with different biochemical parameters.**
Methods: **A total of 505 pregnant women (180 with T2DM, 160 GDM patients, and 165 controls) were enrolled in the current study. SHBG, ILGF-1, and fibronectin were estimated by using the ELISA technique.**
Results: **The GDM and T2DM groups had higher ILGF-1 and fibronectin levels than the control group, while having a lower SHGB level. The correlations of clinical characteristics with ILGF-1, SHBG, and fibronectin showed that ILGF-1 in GDM patients was positively associated with HbA1c% and insulin. T2DM was positively related to insulin and insulin resistance, as well. There was a positive association between SHBG and insulin among the T2DM groups. Furthermore, in T2DM individuals, fibronectin was positively related with HbA1c% and glucose.**
Conclusions: **The study suggests that the circulating levels of fibronectin, SHBG, and ILGF-1 are linked to GDM and T2DM risk. Hence, the circulating concentrations of these biomarkers are potentially useful for predicting the risk of GDM as well as developing T2DM.**
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KEYWORDS

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INTRODUCTION

Gestational diabetes mellitus (GDM) is characterized as a variable-severity glucose intolerance identified in the second or third trimester of pregnancy in patients who have never been diagnosed with diabetes before [1]. About 15% of women are estimated to be affected by GDM, and it is considered one of the most common pregnancy problems [2]. Comparable to type 2 diabetes mellitus (T2DM), its prevalence has been rising globally in accordance with lifestyle modifications, the growing rate of obesity, and the aging of pregnant women [3]. Advanced maternal age, obesity and overweight, inactivity, family history of type 2 diabetes, previous

pregnancy-onset GDM, arterial hypertension, and polycystic ovarian syndrome are the most prevalent risk factors [4].

GDM is linked to a higher risk of short- and long-term issues for the mother and the developing fetus. Newborns are more likely to suffer from respiratory distress syndrome, macrosomia, neonatal hypoglycemia, and neonatal jaundice in the short term [5]. Long-term risks of obesity, type 2 diabetes, cardiovascular diseases, related metabolic disorders, and neuropsychiatric morbidities are higher for neonates born from complicated GDM pregnancies [6]. The risks of preterm birth, preeclampsia, and cesarean delivery are higher in women with GDM. Over time, GDM significantly raises the chance of getting T2DM in the first five years after giving birth, and it elevates the lifetime risk of getting diabetes to 60% [7].

The entire etiology of GDM remains currently unclear. Nonetheless, significant influencing factors include a beta-cell malfunction and the inability of insulin production to overcome the elevated insulin resistance that resulted from the pregnancy [8]. In order to further clarify the pathophysiology of GDM, many studies concentrate on searching for novel risk factors for this disorder, other than maternal, which could also define prognostic variables for the progression of type 2 diabetes after birth [8].

Insulin-like growth factors (ILGFs) have been linked to GDM and its problems for mothers and newborns. They also play a role in growth and metabolism regulation, insulin sensitivity, and glucose metabolism [9]. So far, clinically it has been observed that insulin-like growth factor-1 (ILGF-1) may change significantly during the duration of pregnancy, accompanied by changes in carbohydrate indexes if the mother has GDM. However, few studies have been carried out for the relevance between the ILGF-1 and carbohydrate indexes [10].

Fibronectins are large dimeric glycoproteins of connective tissue with a molecular weight of 450 kDa [11]. The levels of fibronectin are varied between different types of diabetes. This proposes that fibronectin may contribute to the onset and progression to issues associated with diabetes [12]. Proinflammatory cytokines have been linked to an increased fibronectin production, as well as the release of extracellular matrix and cell surface fibronectins into the circulation [13]. It has been demonstrated that these vascular tissue alterations start early in pregnancy and occur even before clinical symptoms appear [11].

Sex hormone-binding globulin (SHBG) is a homodimeric glycoprotein that circulates in the bloodstream and has a high affinity for binding androgens and estrogens while controlling their bioavailability [14]. New research indicates that T2DM is linked to low SHBG levels [15]. In women, low levels of SHBG are linked to hyperglycemia, compensatory hyperinsulinemia, and insulin resistance [16]. The exact relationships of circulating fibronectin, SHBG, and ILGF-1 with DM and GDM remain inconsistent. Therefore, in this study we evaluate the association between these biomarkers in DM and GDM. Additionally, we evaluate their correlations with different biochemical parameters.

MATERIALS AND METHODS

Subjects

The study was conducted on 505 pregnant women (160 with GDM, 180 with T2DM, and 165 healthy non-diabetic controls), at the Reproductive Health and Family Planning Department of the Medical Research Center of Excellence (MRCE), the National Research Center, Egypt. The GDM diagnosis was performed according to the International Association of Diabetes and Pregnancy Study Groups [17]. Diagnosis of type 2 diabetes was identified based on the clinical practice guidelines provided by the American Diabetes Association [18].

Pregnant women with type 1 diabetes mellitus, preeclampsia, thyroid disorders acute or chronic renal disease, liver disease, cardiovascular disease, or poly cystic ovary syndrome were excluded.

Measurements

Age and BMI were documented. Participants' blood samples were obtained in non-heparinized tubes and were taken some time between the 24th and 28th week of pregnancy. The blood samples were then separated for the evaluation of the biochemical parameters and the examination of ILGF-1, SHGB, and fibronectin, after being centrifuged for 10 minutes at 3,000 rpm within 20 minutes of the blood draw.

Biochemical analyses

Stanbio Laboratory, USA, was used to assess the levels of glycosylated hemoglobin A1c (HbA1c) and fasting plasma glucose (FPG). Using kits from Immunospec, enzyme-linked immunosorbent assays (ELISA) were used to measure the levels of human serum insulin. The formula for calculating the model assessment of insulin resistance (HOMA-IR) was as follows: fasting insulin (μU/L) x fasting glucose (mg/L)/22.5 [19].

A quantitative sandwich enzyme-linked immunoassay (ELISA) method was used to assess SHBG. The kit for SHBG analysis was supplied by IBL International GmbH, Germany. ILGF-1 and fibronectin were estimated by using the ELISA technique from Wahan EIAab Science, China, consistent with manufacturers' instructions.

Statistical analysis

Data analysis was done by using SPSS for Windows, version 20. Data showed as mean ± SD. The mean differences between the groups were compared by ANOVA. Person's correlation analyses were used to calculate the degrees of association between the continuous variables. The abilities of the ILGF-1, SHGB, and fibronectin values to detect GDM or T2DM were tested with the receiver operating characteristic (ROC) curve.

RESULTS

The clinical characteristics of the participants are summarized in Figure 1. The three groups were matched in age (all $p > 0.05$). BMI, Glucose, HbA1c, insulin, and HOMA-IR levels were higher in both GDM and DM groups, as compared to the controls ($p < 0.05$).

The levels of circulating ILGF-1, SHGB, and fibronectin in the three groups are summarized in Table 1. The GDM and T2DM groups had higher ILGF-1 and fibronectin levels than the control group, while having a lower SHGB level ($p < 0.001$).

The correlation between the clinical characteristics and ILGF-1 is represented in Table 2. GDM was positively associated with HbA1c% and insulin. T2DM was positively related to insulin and insulin resistance, as well. Table 3 shows the correlation between the clinical parameters and SHBG. There was a positive association between T2DM and insulin. The correlation between fibronectin and clinical variables is revealed in Table 4. T2DM was positively related with HbA1c% and glucose.

The predictive accuracies of ILGF-1, SHGB, and fibronectin as probable markers for GDM and T2DM were determined with the receiver operating characteristic curve (ROC) analyses (Figures 2, 3, and 4, respectively).

DISCUSSION

GDM is a common complication during pregnancy, which imposes a great threat to the health and safety of both the mother and the baby [20]. A pregnant woman with GDM has a high risk of type 2 diabetes [21]. Therefore, the timely diagnosis and treatment of GDM is of great importance to a pregnant woman [22]. One of the main objectives for researchers in the field of maternal-fetal medicine is the identification of potential biomarkers for the early detection of women who are predisposed to develop GDM. These biomarkers could either replace the current methodology or increase the predictive value of current prenatal screening. As a consequence, the current research has mainly focused on the relevance between the SHBG, fibronectin, and ILGF-1 levels of the pregnant woman with GDM and T2DM to provide some data for clinical prevention, diagnosis, and treatment of this disease.

In the present research, it was found that the SHBG level has a strong inverse relationship with the risk of gestational diabetes and T2DM. This relation has been documented in other research to be significantly stronger [16,23,24]. Moreover, in previous studies, a reduced SHBG level in the early stages of pregnancy has been related to subsequently developing GDM [16,23,24]. Regarding to the SHBG levels in T2DM, in studies by Ding et al., conducted in 2006 and 2009 on a number of early-diagnosed T2DM patients and a similar number of healthy controls, the elevated serum SHBG concentrations were linked to a lower incidence of type 2 diabetes [25,26].

The current findings give insight into the early stages of the diabetes cascade and improve our knowledge of the various roles of SHBG in relation to the type and level of hyperglycemia during pregnancy. The findings also imply that SHBG may be useful in identifying women who are at high-risk for developing GDM at an early stage, which may enable an earlier and more precise diagnosis and treatment.

After pregnancy, up to 50% of women with GDM develop a glucose metabolism issue. Women who have developed fasting hyperglycemia, early-onset, or medically managed disorders are more susceptible to suffering from type 2 diabetes subsequently [27]. According to certain explanations, SHBG may have a significant impact on the pathophysiology of T2DM by regulating the actions of sex hormones [26]. Therefore, we propose that the lower levels of SHBG detected in women with more severe GDM, particularly in those whose condition started at early stages and were treated with medication, may indicate a more advanced stage in the diabetic cascade.

In our study, correlation analyses in the T2DM group showed that insulin and homeostasis model assessments of insulin resistance (HOMA-IR) were positively associated with SHBG. Otherwise, a negative correlation was found in the GDM group. In accordance with another investigation, there was a greater association between SHBG and insulin resistance in T2DM [28]. Whereas Joyce et al. demonstrated that SHBG is inversely associated with insulin resistance in diabetes [29]. The inconsistent results could be attributed to various disease populations, ethnicities, and enrolled subject sample sizes. Furthermore, there may be some variation in the approaches used to evaluate insulin sensitivity between the studies. Still unclear though are the precise pathways that link insulin resistance to SHBG. An *in vitro* investigation revealed that SHBG might reduce inflammation, which may act as a mediator for SHBG's preventive impact against the development of metabolic syndrome [30].

Based on the study results, the circulating levels of fibronectin were increased in both gestational and type 2 diabetic patients compared to the control non-diabetic individuals. In line with a recent report, Kanta et al. [31] found an increase in the fibronectin concentration in the diabetic kidney patients. Fibronectin is a prominent component of plasma and as a result of this fibronectin accumulation is associated with diabetes. Furthermore, research suggests that elevated glucose levels may lead to a fibronectin overexpression [32].

The correlation analysis of the present study showed that the circulating fibronectin levels were significantly related to plasma glucose and HbA1c in T2DM patients. On the other hand, the fibronectin did not significantly change between the T2DM and non-DM subjects [12]. In addition to prior research, Huhn et al. found no discernible variation in the fibronectin levels between the

Table 1. Serum levels of ILGF-1, SHGB, and fibronectin in the studied groups.

	Controls (n = 165)		Gestational Diabetes (n = 160)		Type 2 Diabetes (n = 180)		p
	Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI	
ILGF-1 (pg/mL)	7.04 ± 1.6	6.79 - 7.29	19.4 ± 3.5	18.89 - 19.98	21.3 ±2.8	20.85 - 21.68	0.000
SHGB (nmol/L)	64 ± 9.3	62.4 - 65.3	33 ± 4.1	32.4 - 33.7	19 ± 3.4	18.5 - 19.5	0.000
Fibronectin (ng/mL)	3.58 ± 1.4	3.37 - 3.79	14.3 ± 1.9	13.99 - 14.61	12.79 ± 1.9	12.5 - 13.1	0.000

Table 2. Correlations between the ILGF-1 concentrations and the studied variables.

	Gestational Diabetes (n = 160)		Type 2 Diabetes (n = 180)	
	r	p	r	p
Glucose (mg/L)	-0.001	0.989	0.117	0.119
HbA1c%	0.179 *	0.024	0.064	0.391
Insulin (µU/L)	0.153 *	0.05	0.301 **	0.000
HOMA-IR	-0.144	0.07	0.209 **	0.005

*** Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.**

Table 3. Correlations between the SHBG concentrations and the studied variables.

	Gestational Diabetes (n = 160)		Type 2 Diabetes (n = 180)	
	r	p	r	p
Glucose (mg/L)	-0.007	0.927	-0.074	0.323
HbA1c%	-0.069	0.388	-0.048	0.519
Insulin (µU/L)	-0.123	0.123	0.235**	0.002
HOMA-IR	-0.029	0.712	0.135	0.071

**** Correlation is significant at the 0.01 level.**

Table 4. Correlations between the fibronectin concentrations and the studied variables.

	Gestational Diabetes (n = 160)		Type 2 Diabetes (n = 180)	
	r	p	r	p
Glucose (mg/L)	0.04	0.612	0.153 *	0.041
HbA1c%	-0.021	0.797	0.311 **	0.000
Insulin (µU/L)	0.101	0.209	-0.041	0.584
HOMA-IR	0.123	0.12	-0.068	0.366

*** Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.**

non-DM controls and the overall number of diabetic patients. This finding implies that fibronectin may not be a major cause of diabetes, but it does not rule out the possibility that abnormalities on fibronectin tissue components may exist among individuals with diabetes [33]. Regarding gestational diabetes, our data are inconsistent with Nagalla et al., who reported an elevated serum level of fibronectin in the first trimester maternal before hyperglycemia occurred [34]. Moreover, the concentration of fibronectin elevated with the progression of pregnancy, which is consistent with previous findings for Mexican pregnant women [35]. Conversely, Paneva-

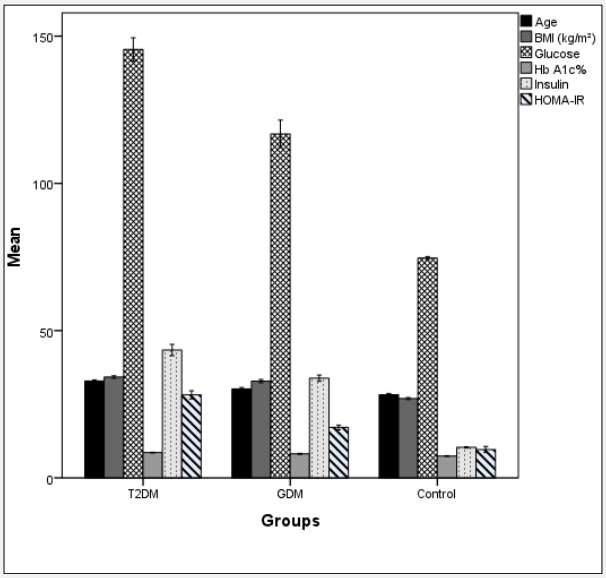
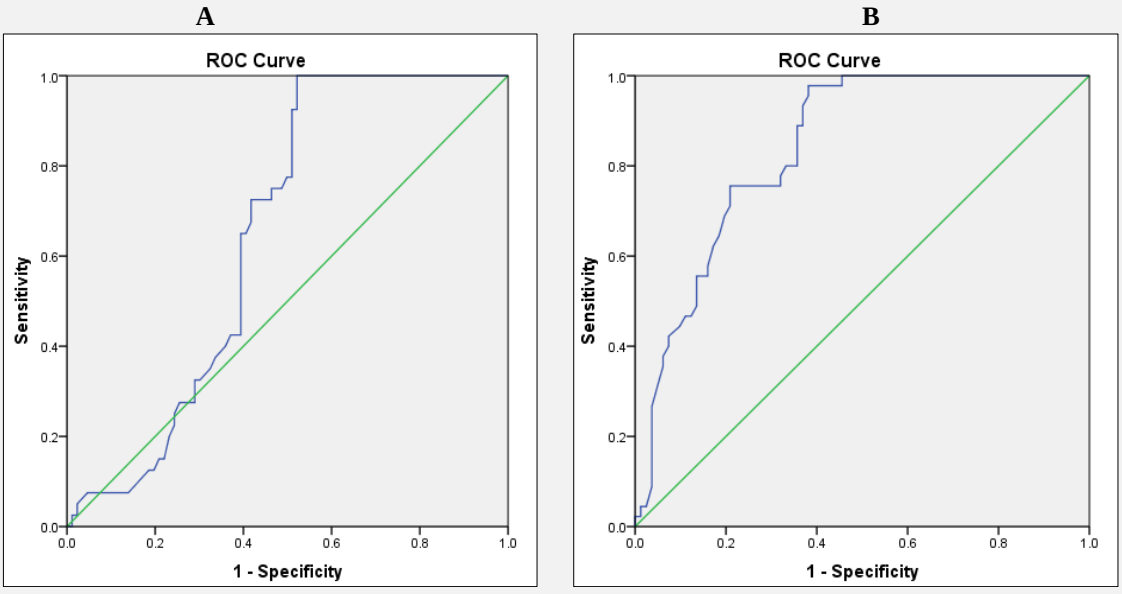
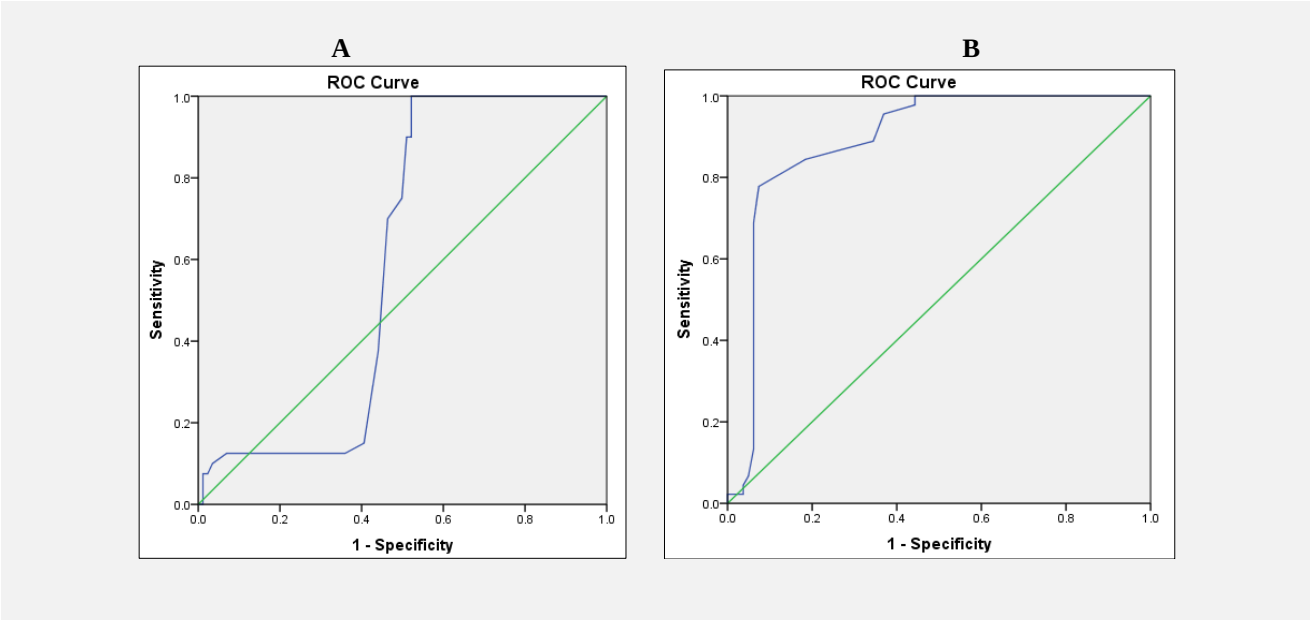


Figure 1. Baseline characteristics of the studied groups.



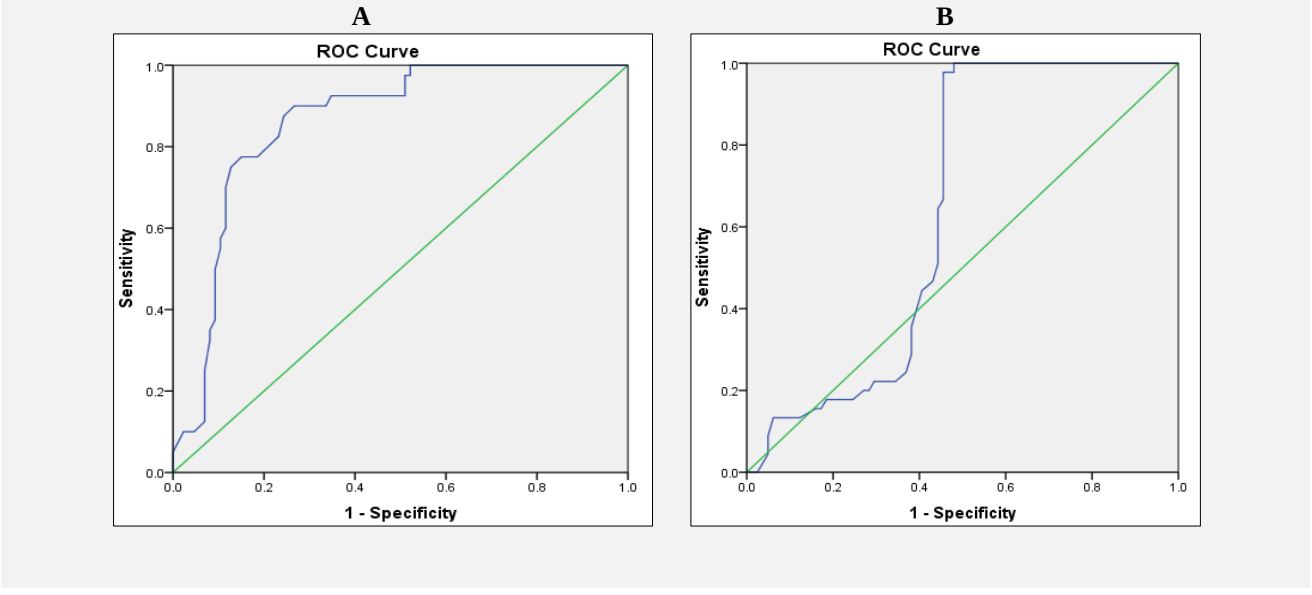
Area under the curve					Area under the curve				
Area	Se	p-value	95% confidence interval		Area	Se	p-value	95% confidence interval	
			Lower bound	Upper bound				Lower bound	Upper bound
0.65	0.024	0.000	0.601	0.694	0.84	0.017	0.000	0.807	0.873

Figure 2 (A). ROC showing the predictive accuracy of ILGF-1 for GDM.
Figure 2 (B). ROC showing the predictive accuracy of ILGF-1 for T2DM.



Area under the curve					Area under the curve				
Area	Se	p-value	95% confidence interval		Area	Se	p-value	95% confidence interval	
			Lower bound	Upper bound				Lower bound	Upper bound
0.6	0.026	0.001	0.543	0.644	0.9	0.015	0.000	0.862	0.921

Figure 3 (A). ROC showing the predictive accuracy of SHBG for GDM.
Figure 3 (B). ROC showing the predictive accuracy of SHBG for T2DM.



Area under the curve					Area under the curve				
Area	Se	p-value	95% confidence interval		Area	Se	p-value	95% confidence interval	
			Lower bound	Upper bound				Lower bound	Upper bound
0.86	0.017	0.000	0.828	0.893	0.64	0.025	0.000	0.591	0.688

Figure 4 (A). ROC showing the predictive accuracy of Fibronectin for GDM.
Figure 4 (B). ROC showing the predictive accuracy of Fibronectin for T2DM.

Masin et al. observed no noticeable variations in pregnant Macedonian women at the 18th, 23rd, 32nd, and 36th week of gestation [36]. This finding could be explained as the first-trimester pregnant women's fibronectin concentrations were considerably lower than those of non-pregnant women. Nonetheless, the levels in the second and third trimesters appeared like those of non-pregnant women. Consequently, in pregnant women, the fibronectin raised as the gestational age increased [37]. This implies that the best time to evaluate fibronectin for disease prediction is between 18 and 24 weeks of gestation or in the middle of the pregnancy. The follow-up for pregnant women revealed that women with greater plasma fibronectin levels had a greater probability of developing GDM than those with lower values.

Our data represented the relation of insulin-like growth factor-1 (ILGF-1) with T2DM and GDM risk. The results showed that ILGF-1 was elevated in T2DM and GDM, compared with healthy controls. Additionally, the correlation of ILGF-1 with insulin among T2DM and GDM was sufficient to support our current evidence. In the same line, our earlier study demonstrated that ILGF-1 polymorphism was linked to the risk of GDM and T2DM, thus it might serve as a biomarker to identify the pregnant women who are susceptible to gestational diabetes [37]. Similarly, a study, carried out by Fan et al., revealed that the secretion of ILGF-1 increases with the elevation of blood glucose in pregnant women with GDM [22]. Anderlova et al. observed unchanged ILGF-1 levels in woman with GDM [9], contrary to the abovementioned findings.

CONCLUSION

In conclusion, our study suggests that the circulating levels of fibronectin, SHBG, and ILGF-1 are linked to T2DM and GDM risk. Hence, these indicators have the potential to enhance an early prenatal GDM screening, provide more insight into the pathophysiological mechanisms underlying the disorder, and make it easier to assess early treatment interventions intended for reducing both the short- and long-term adverse effects for the mother as well as for the newborn. It is crucial to inform the women, whose pregnancies were affected by GDM, that they are more likely to develop T2DM and GDM in later pregnancies. Along with pharmacological therapies, encouraging these women to undergo dietary and lifestyle changes may help lower their vulnerability of developing GDM in their subsequent pregnancies or T2DM later in life.

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Declaration of Interest:
The authors declare that they have no conflicts of interests.

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