Original Article

High Serum Levels of Afamin and Tumor Necrosis Factor-α during The First Trimester might be used as Early Predictors for Gestational Diabetes Mellitus in Euglycemic Pregnant women

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ABSTRACT

Objectives: Evaluation of serum levels of visfatin, tumor necrosis factor-α (TNF-α), afamin, and fetuin-A in euglycemic women at the time of pregnancy diagnosis (Booking time) as early discriminators for women vulnerable to develop gestational diabetes mellitus (GDM).

Patients and Methods: 150 euglycemic newly pregnant women were clinically evaluated and gave blood samples at booking time and at the 24th gestational week (GW). Glucose intolerance was diagnosed using the 75-gm oral glucose tolerance test (OGTT), glycemic control state was checked by the level of glycated hemoglobin (HbA1c), and insulin resistance (IR) was diagnosed using the homeostasis model assessment of IR (HOMA-IR) score. Serum levels of the studied biomarkers were estimated at-booking time and at the 24th GW. Group IR/GDM included women who developed IR and/or GDM, group IS/NG included women who continued their pregnancy free of IR or GDM.

Results: At the 24-GW, all women had higher blood glucose (BG) and HbA1c levels, and HOMA-IR index in comparison to their booking levels, but the difference was significant for women of IR/GDM group and 22 women (14.7%) developed GDM, while 58 women (38.7%) developed IR. At the 24th GW, serum levels of the four biomarkers were significantly higher in blood samples of women of IR/GDM group in comparison to their at-booking levels and to corresponding levels of women of IS/NG group. Statistical analyses defined high at-booking serum TNF-α and afamin levels as early predictors for uncontrolled BG levels, while high TNF-α, visfatin, and afamin serum levels are the significant predictors for HOMA-IR of >2 at the 24th GW.

Conclusion: Elevated serum levels of the studied biomarkers early in pregnancy may play a role in the development of GDM. High serum TNF-α and afamin might be used as early discriminative biomarkers for women liable to develop GDM and/or IR later in pregnancy.

Key Words: Afamin, euglycemic women, fetuin A., gestational diabetes mellitus, tumor necrosis factor-α, visfatin.

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INTRODUCTION

Gestational diabetes mellitus (GDM), a common or the commonest pregnancy-associated complication[1] that harms the health of pregnant women and affects fetal/infant outcomes during and after pregnancy[2]. Women with a history of GDM are at a higher risk of developing postpartum type 2 diabetes mellitus[3]. GDM alters fetal autonomic control even if the metabolic adjustment is comparable to healthy controls[4], increases the risk of fetal heart malformations[5], and is associated with a high incidence of large-for-gestational-age infants despite close monitoring of glucose levels[2].

Pathogenesis of GDM is still uncertain, MicroRNA-362-5p dysregulation in the placenta is involved in GDM[1] and long noncoding RNAs are related to the pathogenesis of recurrent pregnancy loss, preeclampsia, and GDM[6]. High maternal body mass index (BMI) and glycaemia increased the production of different placental docosahexaenoic acid, which has an impact on different metabolic pathways and is associated with GDM and fetal macrosomia[7].

Cytokines are small proteins (~5-20 kDa) released by cells and are involved in autocrine, paracrine, or endocrine signaling. Cytokines are characterized by pleiotropy, redundancy, being produced in a cascade, act synergistically or antagonistically, and are mostly named according to the cell of origin[8]. Hepatokines are liver-derived signaling protein molecules that are implicated in glucose and lipid metabolism[9]. Secretion of hepatokines is regulated by metabolic stressful conditions, either physiological as fasting or exercise[10] or pathophysiological as obesity[11] and insulin resistance[12].

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Adipocytokines are a family of cytokines and hormones that are primarily secreted by different cells that make up white adipose tissue[13]. Adipocytokines function by paracrine and endocrine activities to facilitate nutritional status cross-talk between several organs such as the liver, heart, skeletal muscle, and brain[14]. Adipocytes also have pro- and/or anti-inflammatory effects and excessively secreted pro-inflammatory adipocytokines may underlie the development of metabolic syndrome that accompanies obesity and insulin resistance[15].

Hypothesis

This study hypothesized that the development of GDM is a possible result of the interaction between already disturbed serum levels of hepatokines and adipocytokines.

OBJECTIVES

Estimation of serum levels of visfatin, adipocytokine, tumor necrosis factor-α, afamin, and fetuin-A in newly pregnant women at the time of pregnancy diagnosis may help to discriminate non-diabetic women vulnerable to develop GDM.

Setting

Obstetrics and Gynecology Department, Faculty of Medicine, Benha University

Design

Prospective observational comparative double-blinded study

Ethical consideration

The study protocol was approved by the Local Ethical Committee at Benha Faculty of Medicine. The enrolled women must sign their consent to attend the outpatient clinic for follow-up of pregnancy progress and to give blood samples for required investigations. Blindness means that enrolled women will be blinded about the type of investigations and the obstetrician will be blinded about the levels of the studied cytokines and the biochemist will be blinded about the indication for studying these parameters and about the demographic data of studied women.

PATIENTS AND METHODS

All women attending the antenatal care unit at Benha University Hospital to assure of being pregnant will be eligible for evaluation. Chemical pregnancy was assured clinically and by detection of the gestational sac by the US. At the time of diagnosis of pregnancy (Booking time), the following items were evaluated age, weight, height for calculation of body mass index (BMI) as weight (kg) divided by height (m^2), family or history of diabetes mellitus, history of GDM for multigravida, number of previous pregnancies, labors and living offspring, mode of delivery for the previous pregnancies, history of pregnancy-associated complications as preeclampsia, preterm birth, small-for-gestational-age, or macrosomia, and estimation of baseline systolic (SBP) and diastolic (DBP).

Exclusion criteria

Multiple pregnancy, history of previous GDM or current DM or even family history of DM, endocrinopathy, BMI >35 kg/m2 at booking time, cardiac, renal or hepatic diseases, refusal to sign the informed consent to attend the follow-up visits, being missed or refusal to give blood samples at the 24th GW.

Inclusion criteria

Women with singleton fetus and free of exclusion criteria were included in the study.

Groups

Study participants were divided according to results of determination of HOMA-IR score and OGTT at the 24th GW into:

1. **Group IR/GDM:** included women whose HOMA-IR score and/or results of OGTT at the 24th GW showed significant difference on comparison to levels estimated at booking time

2. **Group IS/NG:** included women whose HOMA-IR score and results of OGTT determined at the 24th GW showed non-significant differences on comparison to their at-booking levels.

Clinical parameters

1. Diagnosis of glucose intolerance: using the 75-gm oral glucose tolerance test (OGTT) which entails estimation of fasting blood glucose (FBG) for women fasted for at least 6-hr and then to re-estimate BG 2-hr after having a snack of 75-gm glucose to determine the 2-hr postprandial blood glucose (PPBG). The OGTT was performed at booking time and 24th gestational week (24-GW). Diagnosis of GDM depended on the results of the OGTT, which were interpreted according to the recommendations of the international association of diabetes and pregnancy study groups as follows: FBG ≥92 mg/dl, and 2-h PPBG ≥153 mg/dl[16].

2. Glycemic control state: The level of glycated hemoglobin (HbA1c) was estimated at booking time and at 24-GW to determine the extent of control of blood glucose and women were categorized accordingly as non-diabetics (HbA1c at a range of 4-6%), pre-diabetics state (HbA1c at a range of 6-6.5%), good diabetic control (HbA1c at a range of 6.5-8%) and diabetics who need interference to achieve control (HbA1c at a range of >8%)[17].

3. Diagnosis of insulin resistance (IR) using the homeostasis model assessment of IR (HOMA-IR) score, which was calculated according to the formula: fasting serum insulin (μU/ml) x [FBG (mg/ml)/18])/22.5; HOMA-IR score of >2 is considered abnormal[18]. HOMA-IR score was determined twice at booking time and 24-GW.

Laboratory investigations

Blood sampling and processing

Two blood samples were obtained, at booking time and 24-GW, under complete aseptic conditions from the antecubital vein. Blood samples were divided into two parts: the 1st was collected into a fluoride-containing tube for estimation of BG and the 2nd part was put in a plain tube, allowed to clot, centrifuged at 1500×g for 15 min and the serum samples were collected in clean Eppindorff tube and stored at -20oC for ELISA estimation of human visfatin, adiponectin, tumor necrosis factor-α (TNF-α), afamin and fetuin-A

Investigation

1. Human visfatin level using ELISA kit (catalog no. ab264623, Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique[19].

2. Human TNF-α was measured with the enzyme-linked immunoassay (ELISA) kit (catalog no. ab46087, Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique[20].

3. Human afamin level using ELISA kit (catalog no. ab114886, Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique[21].

4. Human fetuin-A was measured with the enzyme-linked immunoassay (ELISA) kit (catalog no. ab108855, Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique[22].

Sample size

Previous studies[23,24] detected significant differences in HOMA-IR score and result of OGTT between pregnant non-diabetic women who developed GDM or IR (30 and 55 women, respectively) compared to those completed their pregnancy with minimal alterations of blood glucose levels (30 and 50 women, respectively). Sample calculation for participant of the current study showed that the study must include a minimum of 58 women per group to get significant difference with study power of 89% and α value of 0.05 and β value of 0.2.

Statistical analysis

The obtained data were presented as mean, standard deviation (SD), numbers, and percentages, median and interquartile ranges (IQR). Parametric data were compared using paired t-test and one-way ANOVA test with Tukey HSD-. Non-parametric data were compared using Chi-square test. Receiver characteristic curve analysis was used to evaluate the ability of studied biomarkers for prediction of development of GDM. Regression analysis of studied biomarkers was used to define the predictors for control of blood glucose levels. Automatic linear model was used to determine the importance of studied biomarkers for discrimination of women liable to develop uncontrolled glucose homeostasis. The Statistical analyses were conducted using the IBM SPSS (Version 23, 2015; IBM, South Wacker Drive, Chicago, USA) for Windows statistical package. *P value* <0.05 was considered statistically significant.

RESULTS

During the study duration, 187 women were eligible for evaluation; 23 women were excluded for not fulfilling the inclusion criteria, and 14 women lost during follow-up, while 150 women attended the 24th GW visit and gave the 2nd blood sample. According to the results of the 24th GW investigations, 22 women got GDM and 58 women became IR for an incidence of 14.7% and 38.7%, respectively and were collected as Group IR/GDM (n=80), while the remaining 70 women were collected as IS/NG group (Figure 1). At booking time, data of women of both groups showed non-significant differences as shown in (Table 1).

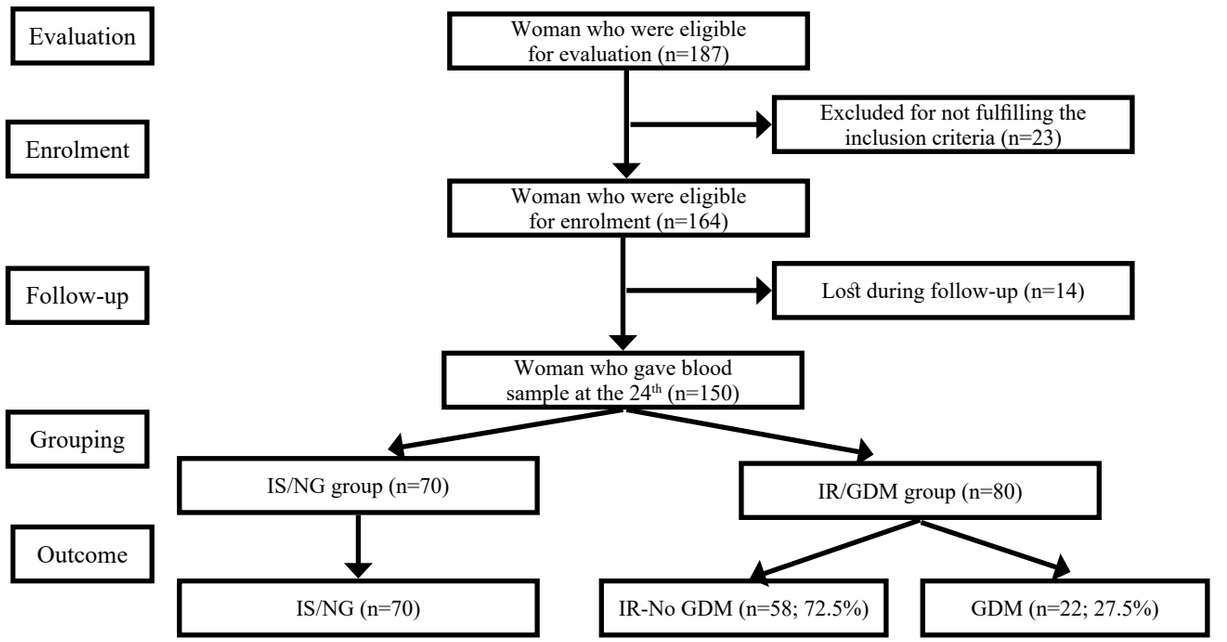

Fig. 1: Flow chart for pregnancy outcome concerning development of IR &/or GDM

Table 1: Enrolment data of women of both study groups

Data	Group	IS/NG (n=70)	IR/GDM (n=80)	*P value*
Age (years)		28.3±3.3	28.6±2.6	0.530
Weight (kg)		83.5±6.7	84.2±4.9	0.454
Height (cm)		169.1±2.6	168.7±2.5	0.344
Body mass index (kg/m2)		29.2±2	29.6±1.9	0.199
Gravidity		2 [1-2]	2 [2-2.75]	0.209
Parity		1 [0-1]	1 [1-1.75]	0.271
Systolic blood pressure (mmHg)		118.4±14.6	116.9±13.9	0.059
Diastolic blood pressure (mmHg)		78.5±10	78.1±9.7	0.592

Data are presented as mean & standard deviation; median & interquartile range [IQR]; P-value indicates the significance of the difference between study and control women; *P-value* >0.05 indicates a non-significant difference

At booking glucose homeostasis data showed non-significant differences between enrolled patients. Estimated BG levels, percentage of HbA1c and HOMA-IR score at the 24th GW of women of IS/NG group showed non-significant differences in comparison to their at-booking data. On contrary, BG levels, percentage of HbA1c and HOMA-IR score estimated at 24-GW were significantly higher in comparison to their at-booking levels and to at 24-GW levels of women of IS/NG group (Table 2).

At booking time, mean serum levels of the studied cytokines showed non-significant differences between patients of both groups. Despite the increased levels of the studied cytokines in samples obtained at the 24th GW of patients of IS/NG group, the differences in comparison to their levels estimated at booking time was non-significant. On the other side, mean levels of studied cytokines were significantly increased in samples obtained at the 24th GW of patients of IR/GDM in comparison both to their levels estimated at booking time and to levels estimated at the 24th GW of patients of group IS/NG (Table 3)

Analysis of at booking time variables as predictors for disturbed glucose homeostasis during pregnancy of non-diabetic women using ROC curve analysis defined high at booking serum levels of afamin, TNF-α, fetuin-A, visfatin, and FBG as the significant predictors with decreasing order of significance (Table 4, Figure 1).

Regression analysis of significant predictors identified by ROC curve analysis defined high serum afamin, TNF-α, visfatin, Fetuin A, and FBG as the significant early predictors for high 24-GW PPBG and development of GDM in non-diabetic pregnant women, and high at booking FBG, serum TNF-α and afamin levels as predictors for uncontrolled glucose blood levels, while high TNF-α, visfatin, and afamin serum levels are the significant variable that can discriminate women liable to develop insulin resistance during pregnancy with high HOMA-IR of >2 at the 24th GW (Tables 5,6).

Table 2: Glucose homeostasis data of women of both study groups

Variable	Group / Time	IS/NG (n=70)	IR/GDM (n=80)	*P value*
FBG (mg/dl)	Booking	84.6±2.3	84.9±3.5	0.538
	24-GW	84.8±4.1	91.9±6.2	<0.0001
	P1 value	0.723	<0.0001	
2hr PPBG (mg/dl)	Booking	121.3±3.4	123.1±7.1	0.056
	24-GW	122.5±4.6	145.4±18.5	<0.0001
	P1 value	0.079	<0.0001	
HbA1c (%)	Booking	4.5±0.35	4.54±0.55	0.566
	24-GW	4.63±0.47	5.3±1.26	0.0001
	P1 value	0.071	<0.0001	
HOMA-IR	Booking	5.77±1.15	6.07±0.95	0.076
Serum insulin	24-GW	6.14±1.2	10.7±1.47	<0.0001
	P1 value	0.062	<0.0001	
	Booking	1.22±0.25	1.29±0.21	0.065
Index	24-GW	1.3±0.26	2.09±0.4	<0.0001
	P1 value	0.058	<0.0001	

Data are presented as mean & standard deviation; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; HbA1c: Glycated hemoglobin A; HOMA-IR: Homeostasis model assessment for insulin resistance; *P-value* indicates the significance of the difference between study and control women; *P1 value* indicates the significance of the difference between booking time and 24th GW measures; *P-value* <0.05 indicates a significant difference; *P-value* >0.05 indicates a non-significant difference

Table 3: Serum biomarkers' levels estimated in blood samples of control and study women

Variables	Group / Time	Control (n=20)	Study (n=150)	*P value*
Fetuin A	Booking	290±95.1	304.5±89.1	0.334
	24-GW	322.5±106	389.4±111.3	0.0002
	P1 value	0.055	<0.001	
	% of change	11.1±3.14	28.8±13.4	<0.001
Visfatin	Booking	25.17±7.8	26.5±8.4	0.318
	24-GW	27.5±9.4	35.67±10.7	<0.001
	P1 value	0.112	<0.001	
	% of change	8.5±4.1	36.3±16.55	<0.001
Afamin	Booking	64.1±17.7	66.86±18.4	0.355
	24-GW	70.01±19.2	83.67±22.65	<0.001
	P1 value	0.061	<0.001	
	% of change	9.3±3.1	26.2±13	<0.001
TNF-α	Booking	2.536±0.78	2.765±0.8	0.079
	24-GW	2.789±0.82	3.289±0.94	0.0007
	P1 value	0.062	0.0002	
	% of change	10.3±2.94	19.5±8.9	<0.001

Data are presented as mean & standard deviation; TNF-α: Tumor necrosis factor-α; *P-value* indicates the significance of the difference between study and control women; *P1 value* indicates the significance of the difference between booking time and 24th GW measures; *P-value* <0.05 indicates a significant difference; *P-value* >0.05 indicates a non-significant difference

Table 4: ROC curve analysis of at booking data and serum biomarkers' levels as early predictors for disturbances of glucose homeostasis during pregnancy in non-diabetic women

	AUC	Std error	*P*	Asymptomatic 95% CI
Afamin	0.873	0.042	<0.0001	0.790-0.956
TNF-α	0.800	0.057	<0.0001	0.688-0.911
Fetuin A	0.770	0.049	<0.0001	0.675-0.865
Visfatin	0.689	0.073	0.005	0.546-0.832
FBG	0.672	0.071	0.010	0.534-0.811

Table 5: Regression analysis of at booking data and serum biomarkers' levels as early predictors for disturbances of glucose homeostasis and development of GDM and IR at the 24th GW of the women of the study group

Variables	GDM		24-GW PPBG		HbA1c		HOMA-IR index	
	β	p	β	p	β	p	β	p
Afamin	0.403	<0.001	0.324	<0.001	0.197	0.008	0.198	0.008
TNF-α	0.349	<0.001	0.287	<0.001	0.255	0.001	0.317	<0.001
Visfatin	0.263	<0.001	0.270	<0.001	Excluded		0.226	0.003
FBG	0.246	<0.001	0.288	<0.001	0.317	<0.001	Excluded	
Fetuin A	0.184	0.002	Excluded		Excluded		Excluded	

GDM: Gestational diabetes mellitus; PPBG: Postprandial blood glucose; HbA1c: Glycated hemoglobin A; HOMA-IR: Homeostasis model assessment for insulin resistance; FBG: Fasting blood glucose; TNF-α: Tumor necrosis factor-α; β: Standardized coefficient; *P-value* indicates the significance of β value; *P-value* <0.05 indicates a significant value; *P-value* >0.05 indicates a non-significant value

Table 6: Automatic linear modeling analysis of the importance of defined variables as predictors for disturbances of glucose homeostasis and development of GDM and IR at the 24th GW of the women of the study group

	24-GW PPBG	HbA1c	HOMA-IR index
Afamin	24%	26%	14%
TNF-α	40%	59%	54%
Visfatin	24%	0	25%
Feutin A	16%	0	7%
BMI	0	15%	0

GDM: Gestational diabetes mellitus; PPBG: Postprandial blood glucose; HbA1c: Glucated hemoglobin A; HOMA-IR: Homeostasis model assessment for insulin resistance; FBG: Fasting blood glucose; TNF-α: Tumor necrosis factor-α; β: Standardized coefficient; *P value* indicates the significance of β value; *P value* <0.05 indicates significant value; *P value* >0.05 indicates non-significant value

DISCUSSION

All studied pregnant women showed pregnancy-associated disturbance of glucose homeostasis parameters (GHP) that was manifested as higher FBG, 2-hr PPBG with loss of control of glucose homeostasis that was evidenced by a high percentage of HbA1c levels at the 24th GW in comparison to the at-booking levels. Moreover, at the 24th GW, 22 women (14.7%) had progressed from simple pregnancy-induced hyperglycemia to GDM.

These findings points to a fact that pregnancy per se is a diabetogenic condition despite being physiological; however, certain women were vulnerable to manifest diabetic BG levels as evidenced by the non-significant differences of glucose homeostasis parameters at the 24th GW in comparison to the at-booking levels in women of IS/NG group. These data go in hand with Li *et al*.[25] who detected significantly elevated BG measures in women who developed GDM than in women who showed progressive increase of their BG measures but did not approach the diagnostic level of GDM and attributed this to progressively declining pancreatic β-cell function as manifested by increased IR scorings. In support of this explanation, the current study detected increased IR in all pregnant women and 58 women (38.7%) had HOMA-IR index higher than the cutoff point for diagnosis of IR, irrespective of progress to GDM or not.

The reported disturbances of GHP at the 24th GW indicated the ability of high levels at booking time to predict oncoming IR and/or GDM; in support of this assumption, Regression analysis defined at-booking FBG as a significant predictor for high 2hr-PPBG, high HbA1c and on coming GDM. Similarly, Immanuel *et al*.[26,27] detected progressively increasing HbA1c levels through pregnancy and estimation of its levels can help to differentiate pregnant women liable to develop GDM or overt diabetes with high specificity for an early HbA1c level of ≥ 5.7%.

On the other side, all pregnant women showed higher levels of studied adipocytokines; visfatin, TNF-α, and fetuin A and levels of the studied hepatokine; afamin at the 24th GW in comparison to levels estimated at booking time, but the differences were non-significant for women of IS/NG group and were significant for women of IR/GDM group. In line with these results, Bawah *et al*.[28] reported significant differences in serum levels of leptin, resistin, and visfatin among women with GDM in comparison to women without GDM and Lu *et al*.[29] detected a role for increased expression of visfatin and fetuin A in the development of GDM. Thereafter, Jin *et al*.[30] reported that the dynamic change in fetuin-A levels was associated with the changes in IR and β-cell function from the 1st to the 2nd trimester, and was associated with an increased risk of the development of GDM. Also, Ramachandrayya *et al*.[31] found the levels of resistin, IL-6 and TNF-α were

higher at 24 than at 12 GW in both healthy pregnant and GDM women with significantly higher levels in women with GDM than women with healthy pregnancy. Recently, Eroğlu *et al*.[32] detected higher serum levels of afamin in women who developed GDM than control women and Al-Musharaf *et al*.[33] detected significantly and progressively increasing serum levels of TNF-α, leptin and resistin from the 1st to the 2nd trimester in GDM women than in no-GDM women and considered these disturbed levels of adipocytokines during the course of pregnancy may play a role in pathogenesis of GDM.

The obtained results and literature review point to a role of disturbed levels of adipocytokines and hepatokines during pregnancy in development of gestational glucose intolerance and the progress to diabetic state. This explanation supported that previously detected by Francis *et al*.[34] and Gęca *et al*.[35] who documented a possible role for disturbed levels of diabetogenic factors secreted by either or both of adipose tissue and hepatocytes in development of GDM.

In support of this suggestion, statistical analyses defined high at-booking serum levels of TNF-α and afamin as the significant early predictors for oncoming GDM and IR during pregnancy with disturbed glucose homeostasis. Similarly, multiple studies[28,29,30,33] documented the early predictability of various adipocytokines for disturbed glucose homeostasis during pregnancy and later development of GDM

Regarding the role of hepatokines as early predictors for pregnancy-induced disturbed glucose homeostasis that was defined statistically for afamin; Cai *et al*.[36] after meta-analysis included 31 observational studies relating hepatokine levels to GDM concluded that measurement of circulating hepatokines in the 1st or 2nd trimesters may improve the identification of women at risk of developing GDM later and Atakul *et al*.[37] documented that estimated serum afamin levels could predict large-for-gestational age fetuses in pregnant women independently of glycemic control status.

In trial to explain the relation between high serum levels of the studied inflammatory cytokine; TNF-α and incidence of GDM among pregnant women, Rakchna *et al*.[38] analyzed genomic DNA for rs2073617 T950C polymorphism and found TNF-α levels were significantly higher in women with CT allele and are independently related to development of GDM. Also, Liu *et al*.[39] analyzed genomic DNA and found TNF-α rs1800629 polymorphism is a risk factor for GDM. As another attribution, Schiattarella *et al*.[40] speculated that inflammatory markers induce endothelium dysfunction and IR and contribute to the pathogenesis of GDM.

CONCLUSION

Pregnancy is a glucogenic condition that was associated with disturbed glucose homeostasis, which may progress in vulnerable women to GDM or at least IR. Elevated serum levels of TNF-α, afamin, visfatin, and fetuin-A early in pregnancy may play a role in the development of GDM. High serum TNF-α, an adipocytokine, and afamin, a hepatokine during the 1st trimester might be used as early discriminative biomarkers for women liable to develop GDM and/or IR later in pregnancy.

LIMITATION

The predictive value of the studied biomarkers needs to be differentiated according to BMI strata.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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