Study of serum zonulin level as an early predictor for gestational diabetes in Egyptian females

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Background
Gestational diabetes mellitus (GDM) is any degree of abnormal glucose tolerance first recognized in the period of pregnancy, and is detected in the second or third trimester of pregnancy. Its incidence has paralleled the epidemic of obesity. It also leads to increasing the risk for glucose intolerance, macrosomia, and development type 2 diabetes mellitus after pregnancy. Many markers were tested for the ability of early diagnosis of GDM. Zonulin is the basic protein that modulates tight junctions to regulate intercellular passage. Zonulin was found to be positively correlated with increased markers of inflammation and with insulin resistance in nonpregnant populations.

Objective
To assess serum zonulin level in pregnant women and to evaluate whether it could be an early predictor for development of GDM.

Patients and methods
The study included 80 pregnant females in the first trimester who were recruited from the obstetrics outpatient clinic at Al-Shatby University Hospital, Alexandria University. Basic data, clinical examination, and laboratory analysis were obtained from all the cases. Serum zonulin level was measured using human enzyme-linked immunosorbent assay kits.

Results
The median level of early serum zonulin level in the females who did not develop GDM was statistically significantly lower than the levels in the females who developed GDM. The best cut-off point to identify the diabetic females in pregnancy was more than 30 ng/ml with 97.22% sensitivity and 100% specificity. There was a highly significant association between the BMI and risk of GDM.

Conclusion
This study revealed that gestational diabetes is a common complication of pregnancy in the middle-aged females. Increasing BMI is associated with high incidence of GDM and high serum zonulin level in the first trimester. It is a sensitive predictor for development of GDM later during pregnancy.

Keywords:
gestational diabetes, insulin resistance, zonulin

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Introduction

The term ‘gestational diabetes’ has been defined as abnormal glucose tolerance that is first recognized during pregnancy [1].

According to WHO preview of studies from 2005 to 2018, the gestational diabetes mellitus (GDM) prevalence is the highest in the Middle East and some North-African countries [2].

The major risk for GDM in pregnancy include personal history of impaired glucose tolerance or GDM in a prior pregnancy [3], positive family history of DM, particularly in relatives of first degree [4], the weight of the female before being pregnant 110% or more than the ideal body weight, major weight gain between pregnancies and in early adulthood [5], or major weight gain during the first 18–24 weeks of pregnancy [6,7]. The age of the pregnant female more than 25 years of age, past history of delivery of a malformed infant or having inexplicable perinatal loss, the pregnant female presenting with passing of glucose in urine at the first prenatal visit, and medical setting/condition related to DM development, such as polycystic ovary syndrome, metabolic syndrome, hypertension, recent glucocorticoid utilization, and multiple gestations [6,7].

Pregnancy is diabetogenic and it is characterized by insulin resistance [8].

The decreased action of insulin in body tissues at normal concentrations of insulin in the plasma is described as insulin resistance. Some factors are related to this, such as abnormal insulin structure, abnormal functioning of its receptors, or abnormal pathway of the signal transduction. The β-cells of pancreas increase the insulin production to compensate for insulin resistance resulting in hyperinsulinemia [9].

β-cells cannot increase insulin production indefinitely. Insulin production declines due to the production of human placental lactogenic by placenta. It is promoting lipolysis, increasing free fatty acids, decreasing maternal glucose uptake, and increasing gluconeogenesis. The condition progresses to glucose intolerance and subsequently to diabetes mellitus [10].

Maternal insulin resistance results in high-fat utilization by the mother more than carbohydrates for production of energy. The maternal glucose transfer through the placenta to the fetus is buffered by utilization of the placental glucose and the insulin secretion by the developing pancreas of the fetus promotes the utilization of glucose among the tissues that are sensitive to insulin (liver skeletal muscle, adipose tissue, and heart). Fetal mass and therefore the need of glucose increases during late gestation [11].

As pregnancy advances, the serum levels of progesterone rise and are related to high inhibition of translocation of GLUT4 and uptake of glucose that are induced by insulin. There are also high estrogen concentrations in pregnancy and 17β-estradiol diminishes insulin sensitivity at high concentrations [12].

There is a distinctive difference between the insulin resistance seen in pregnancy and that associated with type 2 diabetes mellitus (T2DM) in that the former is of short duration. Women who develop GDM are a more insulin-resistance group than women who remain glucose tolerant [13].

GDM is diagnosed by one of two sets of diagnostic criteria as one-step strategy: performs a 75-g oral glucose tolerance test (OGTT), with measurement of the levels of glucose in plasma when a patient is fasting for at least 8, 1, 2 h, and at 24–28 weeks of gestation in females not previously suffering from DM. The GDM diagnosis is confirmed in the condition of any of the next values of glucose in plasma that are reached: fasting more than or equal to 92 mg/dl, 1 h more than or equal to 180 mg/dl, or 2 h more than or equal to 153 mg/dl [14].

Zonulin is chiefly released from the hepatic cells, but also can be secreted from adipose tissue, enterocytes, heart, brain, lungs, immune cells, skin, and kidney [15].

Zonulin is a suggested serum marker for the permeability of the intestine, the increased concentrations reflecting an increased intestinal permeability. This has been found to be correlated with the increased markers of inflammation and with insulin resistance in nonpregnant populations [16].

The composition of diet has lately been found to be correlated with the serum levels of zonulin, recommending a novel mechanism by which diet may play a role in the beginning of the metabolic disorders that are correlated with inflammation [17].

This study was conducted aiming to assess serum zonulin level in pregnant women and to evaluate whether it could be an early predictor for development of GDM.

Patients and methods

Study design

This is a prospective observational study that was conducted in Obstetrics Outpatient Clinic at Al-Shat by University Hospital, Alexandria University.

Patients

This study included a total of 100 pregnant females recruited during the first visit during the first trimester. With the follow-up during pregnancy, 20 females did not follow up regularly, so they were dropped out from the study. The total analysis included 80 pregnant females.

We included patients with the following criteria: obese females (BMI≥30), multiparity, history of recurrent miscarriage, hypertension or preeclampsia in the current pregnancy, family history of DM, smoking, and polycystic ovary syndrome. The patients with the following conditions were excluded: pregestational diagnosis of DM, patient with overt diabetes at first visit, history of GDM, autoimmune disorders, hepatic or renal dysfunction (creatinine level >1.5 mg/dl), preeclampsia, active infection, thyroid dysfunction, and patients who were unable/unwilling to provide consent.

Methodology

After obtaining a written informed consent from all the included females, all cases were subjected to complete history taking (demographic data, medical history, drug, and family history) and through full general [body weight, height and BMI, waist circumference, waist–hip ratio (in the first visit), and the vital signs]. Laboratory investigations, including fasting serum glucose, glycosylated hemoglobin (HbA1C), and serum levels of zonulin, were tested during the first visit of the included females. At 24–26 weeks of pregnancy, the GDM diagnosis was done by using an OGTT [18].

A single-stage 75-g OGTT was utilized for pregnant females in the 24–28 weeks of gestation without GDM history. The level of fasting glucose in plasma (at least 8 h of fasting) was measured and followed by giving of 75 g of glucose orally and measuring the first and second hour levels of glucose.

The diagnosis of GDM was based on the following criteria: fasting blood glucose: more than or equal to 92 mg/dl, or first-hour plasma glucose: more than or equal to 180 mg/dl, or second-hour plasma glucose: more than or equal to 153 mg/dl.

Plasma levels of zonulin were measured utilizing the sandwich-enzyme-linked immunosorbent assay (ELISA) method, with the human ELISA kit (Distribuito in ITALIA - da Li StarFishS.r.l.) (Cat. No. BTB-E3704Hu).

This kit is an ELISA. Zonulin is put in the wells precoated with zonulin monoclonal antibody. After incubation, a biotin-conjugated antihuman zonulin antibody is put and binds to human zonulin. After incubation, the unbound biotin-conjugated antihuman zonulin antibody is washed away. Streptavidin–HRP is put and binds to the biotin-conjugated antihuman zonulin antibody. After incubation, the unbound streptavidin–HRP is washed. After that, a substrate solution is put and the development of the color takes place in proportion to the human zonulin amount. The termination of the reaction is carried out by adding of stop solution.

Results

The mean age of the cases included in this study was 27.71±2.89 years with range between 22 and 36 years. Most of the cases (83.8%) were in the age group between 20 and 30 years, while 16.3% of the included females were above 30 years. Among the included females, most of them (86.3%) were in the third month of pregnancy, while 13.8% were in the second month of pregnancy. The mean BMI of the cases in the study was 26.50±2.0 kg/m^2 with range between 22 and 31 kg/m^2. Most of the cases (81.3%) were overweight, 6.3% of the cases were with normal weight, and 12.5% of the cases were obese (Table 1).

As shown in Table 2, the mean level of fasting OGTT in the third trimester was 99.85±23.12 with range between 75 and 160, 53.2% had values less than 92, while 46.3% had values more than or equal to 92. The mean value after 1 h was 171.94±37.05 with range between 91.80 and 255, 55% had values less than 180, while 45% had values more than or equal to

Table 1 Basic criteria of the females included in the study

	n (%)
Age (years)	
20–30	67 (83.8)
>30	13 (16.2)
Mean±SD	27.71±2.89
BMI (kg/m^2)	*n* (%)
Normal weight (18.5–24.9)	5 (6.3)
Overweight (25.0–29.9)	65 (81.3)
Obesity (≥30)	10 (12.5)
Mean±SD	26.50±2.0
Month of pregnancy	*n* (%)
Second month	11 (13.8)
Third month	69 (86.2)
Mean±SD	2.86±0.35

Table 2 Distribution of the studied cases according to oral glucose tolerance test at third trimester and percentage of females with gestational diabetes mellitus

OGTT (mg/dl)	*n* (%)
Fasting	
<92	43 (53.8)
≥92	37 (46.3)
Mean±SD	99.85±23.12
After 1 h	
<180	44 (55.0)
≥180	36 (45.0)
Mean±SD	171.94±37.05
After 2 h	
<153	47 (58.8)
≥153	33 (41.3)
Mean±SD	131.32±27.43
State of GDM	
Diabetic	44 (55)
Nondiabetic	36 (45)

GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test.

180. The mean value after 2 h was 131.32±27.43 with range between 69 and 180, 58.8% had values less than 153, while 41.3% had values more than or equal to 153. According to these results, there were 36 (45%) females diabetic and 44 (55%) females nondiabetic.

As shown in Table 3, the median level of early serum zonulin level in the females who did not develop GD was 7 ng/ml with range between 4.2 and 13 ng/ml, which was significantly lower than the levels in the females who developed GD (median, 58 ng/ml with range between 55.5 and 59.5 ng/ml) (P<0.001).

The best cut-off point to identify the diabetic females in pregnancy was more than 30 ng/ml with 97.22% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 97.8 negative predictive value (NPV). The area under the curve was 1 with a statistically significant value (P<0.001). These data are illustrated in Table 4 and shown in Figs 1–14.

As shown in Table 5, in the group of females with normal weight, there were three (60%) nondiabetic females and two (40%) diabetic females, in the group of females with overweight, there were 42 (64.6%) nondiabetic females and 23 (35.4%) diabetic females, and in the group of obese females, all the females were diabetic (100%). There was a high statistically significant difference between the three groups regarding the incidence of DM (P<0.001).

As shown in Table 6, there was a statistically significant positive correlation between serum zonulin levels with BMI, HbA1C, fasting blood glucose, and OGTT values.

Discussion

GDM is a significant source of mortality and morbidity for both infant and mother [19]. Increased levels of glucose in blood negatively influence the development of the fetus in the uterine life. Furthermore, GDM elevates the possibility of development of DM in the next stages of life [20].

The increased zonulin concentrations reflect an increased intestinal permeability [21]. This has been found to be correlated with the increased markers of inflammation and with insulin resistance in nonpregnant populations [22].

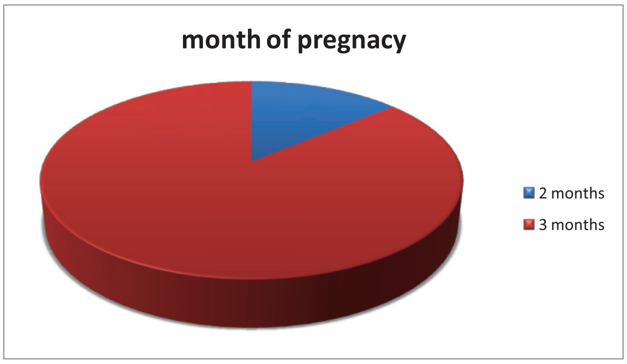
The increased serum levels of zonulin were proposed to be related to the beginning of the metabolic disorders that correlated with inflammation [23].

This study was conducted to assess serum zonulin level in pregnant women and to evaluate whether it could be an early predictor for development of GDM.

This study included 80 pregnant females who were included during their initial visit in the first trimester and followed up till the third trimester to detect the appearance of gestational diabetes.

The mean age of the cases included in this study was 27.71±2.89 years with range between 22 and 36 years.

Figure 1



Distribution of the studied cases according to month of pregnancy (N=80).

Table 3 Comparison between non-diabetes mellitus and diabetes mellitus according to serum zonulin

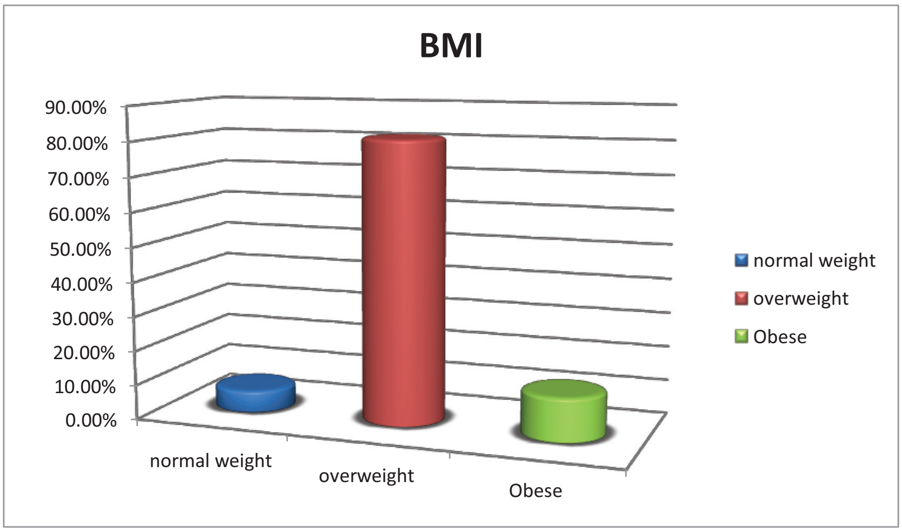
Serum zonulin (ng/ml)	Total (N=80)	Non-DM (N=45)	DM (N=35)	P
Median (range)	15.5 (2.30–65.0)	7 (2.30–30.0)	58 (52.0–65.0)	<0.001*
Mean±SD	30.52±24.75	9.28±6.23	57.83±2.84	

DM, diabetes mellitus. *Serum zinoulin was higher in patients with GDM.

Table 4 Agreement (sensitivity, specificity) for serum zonulin to diagnose diabetes mellitus patients from non-diabetes mellitus

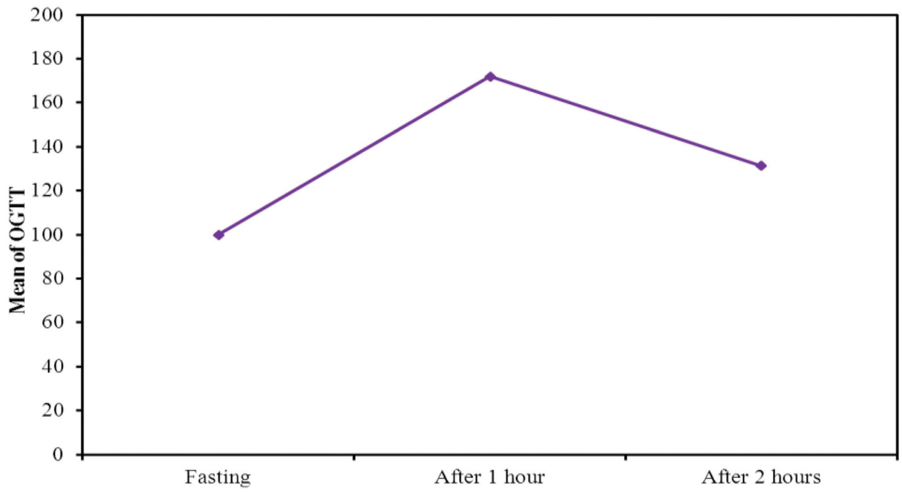
	AUC	P	95% CI		Cut off	Sensitivity	Specificity	PPV	NPV
			LL	UL					
Serum zonulin (ng/ml)	1.000*	<0.001*	1.000	1.000	>30	97.22	100.0	100.0	97.8

Figure 2



Distribution of the studied cases according to BMI (*N*=80).

Figure 3



Distribution of the studied cases according to OGTT (*N*=80). OGTT, oral glucose tolerance test.

Most of the cases (83.8%) were in the age group between 20 and 30 years, while 16.3% of the included females were above 30 years.

The mean females' age in the study conducted by Mokkala *et al.* [17] was 30.1 (4.9) years. While in the study by Demir *et al.* [16], it was 30.2±4.9 years in the control group and 31.3±6.4 years in the GDM females.

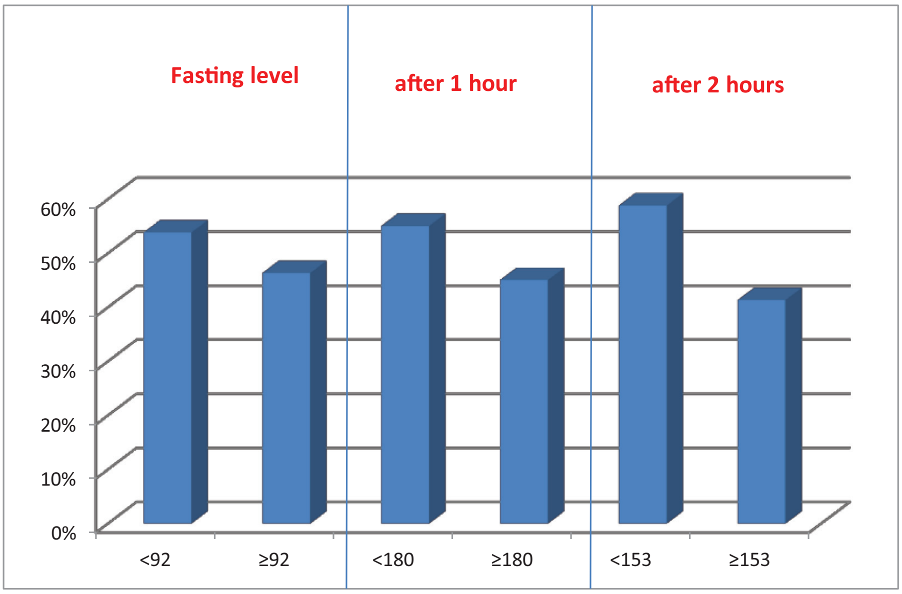
The age of pregnant females more than 30 years was identified as the optimum pregnancy period where the risk of GDM was increased [24]. Similarly, the gestational diabetes prevalence was documented to elevate with advancing age of pregnant females [25,26].

The overweight prevalence is ∼30% in young women at fertility age and thus a relatively large proportion of pregnant females are at an increased risk for GDM [27].

In this study, the mean BMI of the cases in the study was 26.50±2.0 kg/m^2 with range between 22 and 31 kg/m^2. Most of the cases (81.3%) were overweight, 6.3% of the cases were with normal weight, and 12.5% of the cases were obese.

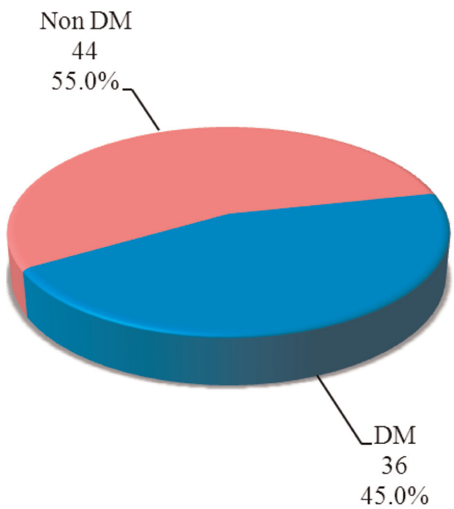
Mokkala *et al.* [17] showed that among the pregnant females included in their study, 43% were overweight (BMI 25–29.99 kg/m^2), 57% were obese (BMI≥30 kg/m^2).

Figure 4



Distribution of the studied cases according to OGTT (*N*=80). OGTT, oral glucose tolerance test.

Figure 5



Distribution of the studied cases according to DM (*N*=80). DM, diabetes mellitus.

Demir *et al.* [16] showed that the mean BMI was significantly different between pregnant females who did not develop GDM and who developed GDM (27.7 ±4.2 and 29.4±4.7 kg/m^2, respectively) (*P*=0.027).

Within the same context, Bawah *et al.* [28], the BMI was significantly higher in the GDMs in comparison with the cases not suffering from GDM.

With the follow-up of the females involved in the study and in accordance with the results of OGTT at 24–26 weeks of gestation, there were 36 (45%) females who developed GDM and 44 (55%) females nondiabetic.

In the report supported by World Diabetes Foundation that revealed among 7141 pregnant women screened for GDM in Upper Egypt in the period between 2014 and 2017, there were 956 women who were diagnosed with GDM.

However, Salem *et al.* [29] conducted a study that included 130 pregnant females screened for the GDM prevalence, and among them, only eight (6%) patients have fulfilled American Diabetes Association criteria to have GDM.

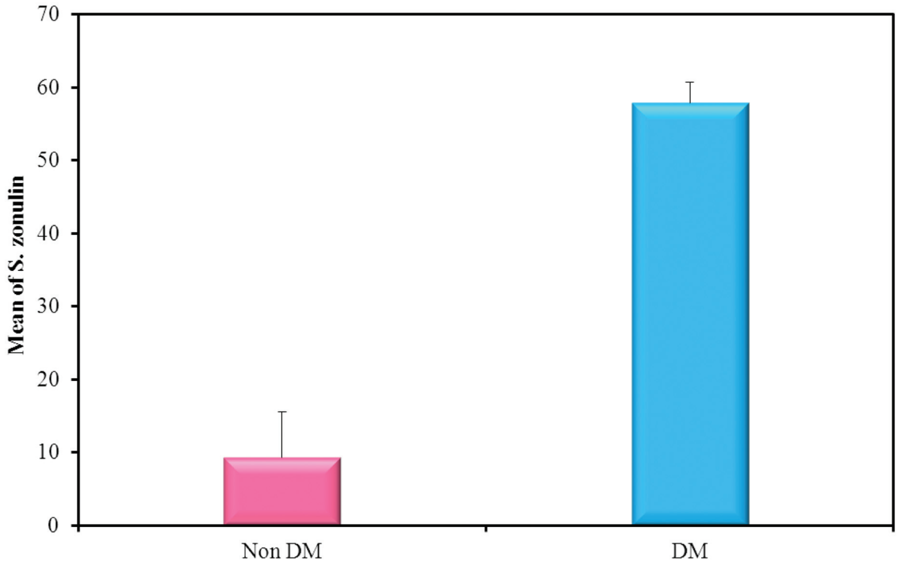
Mokkala *et al.* [17] showed that GDM was diagnosed in 24 (27%) mothers out of 88. Bawah *et al.* [28] reported that a total of 21 out of 314 developed GDM representing 6.7%.

These variations could be explained by the dissimilarities in the social and demographic characteristics of the studied populations, the sample size, and the applied diagnostic criteria. It should also be noted that the global GDM prevalence was documented to be widely variable (1–28%) due to ethnic heterogeneity among different tested populations [30,31].

In this study, the median level of serum zonulin in the nondiabetic females was 7 ng/ml with range between 4.2 and 13 ng/ml, which was majorly lower than the levels in the diabetic females (median, 58 ng/ml with range between 55.5 and 59.5 ng/ml) (*P*<0.001).

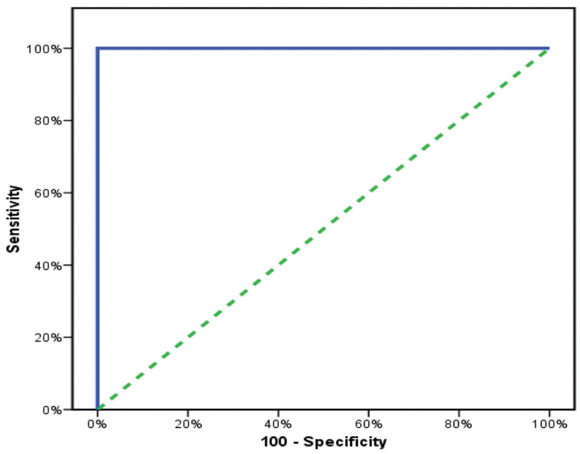
This came in agreement with Demir *et al.* [16] who showed that the mean level of zonulin in the females

Figure 6



Comparison between non-DM and DM according to serum zonulin. DM, diabetes mellitus.

Figure 7



ROC curve for serum zonulin to diagnose DM patients from non-DM. DM, diabetes mellitus; ROC, receiver operating characteristic.

who did not have GDM was 12.8±3.3 ng/ml, which was statistically significantly lower as compared with the females who developed GD (32.6±4.8 ng/ml) (P<0.001).

The result of this current study was comparable with the results of Mokkala *et al.* [17], who showed that the serum levels of zonulin in the first trimester of pregnancy were greater in females who had GDM in the second trimester of pregnancy [mean (SD) 53.4 (14.3) ng/ml (n=16)] in comparison with the cases who did not [45.2 (9.7) ng/ml] (n=64) (P=0.008).

Similar results were revealed by Bawah *et al.* [28], who showed that first-trimester zonulin concentration was significantly higher in those who subsequently developed GDM in comparison with the cases who did not develop the GDM (59.50±9 and 41.84 ±7.21 ng/ml, respectively).

The best cut-off point to identify the diabetic females in pregnancy was more than 30 ng/ml with 97.22% sensitivity, 100% specificity, 100% PPV, and 97.8 NPV. The area under the curve was 1 with a statistically significant value (P<0.001).

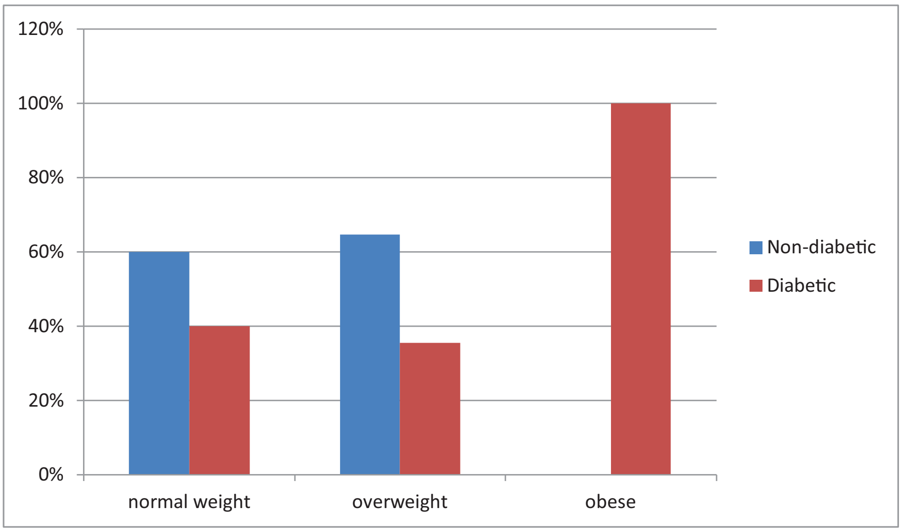
Demir *et al.* [16] have reported that the level of zonulin in plasma more than or equal to 20 ng/ml showed a specificity of 100%, a sensitivity of 98.8%, a NPV of 100%, and a PPV of 98.8%.

Mokkala *et al.* [17] showed that the optimal cut-off value for serum zonulin was more than or equal 43.3 ng/ml, in predicting mid-pregnancy GDM, with a sensitivity of 88% [95% confidence index (CI): 71–100%) and a specificity of 47% (95% CI: 33–58%). This level showed a NPV of 94% (95% CI: 85–100%) and a PPV of 29% (95% CI: 16–41%).

Bawah *et al.* [28] showed that the curve for zonulin indicates a large area under the curve away from the reference line showing its ability to positively predict GDM. The cut-off points of more than 47.5 ng/ml with 80.95% sensitivity, 80.41% specificity, and respectively in predicting GDM, with NPV and PPV of 0.986 and 0.708, respectively.

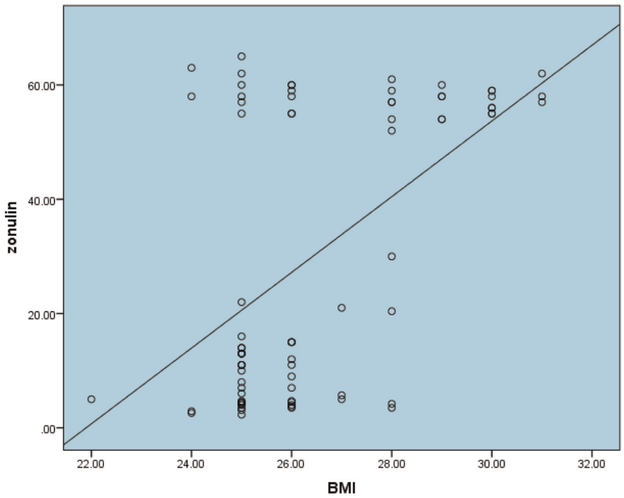
Previously, some other serum markers were assessed for utilization in prediction of GDM in early pregnancy

Figure 8



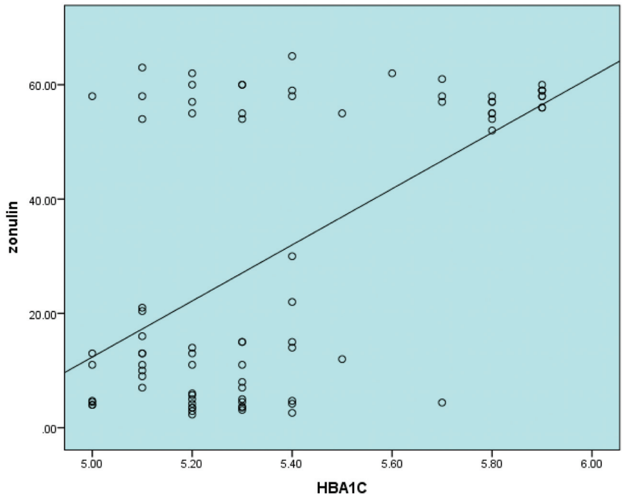
Percentage of diabetic and nondiabetic females according to DM. DM, diabetes mellitus.

Figure 9



Correlation between serum zonulin levels with BMI.

Figure 10



Correlation between serum zonulin levels with HbA1C. HbA1C, glycosylated hemoglobin.

and showed different degrees of success. Fasting plasma glucose measurement had a specificity of 77% and sensitivity of 47 [32].

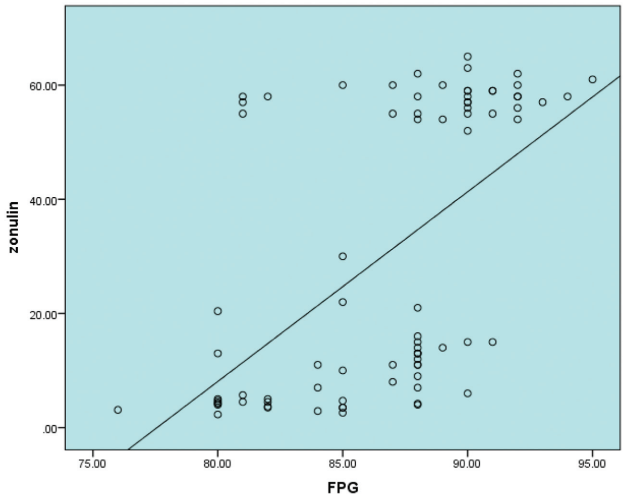
Further, HbA1C showed a specificity of 95% and sensitivity of 19, and fructosamine a specificity of 95% and sensitivity of 12 [32], and high-sensitivity C-reactive protein a specificity of 87% and sensitivity of 37 [33].In this study, in the group of females with normal weight, there were three (60%) nondiabetic females and two (40%) diabetic females, in the group of females with overweight, there were 42 (64.6%) nondiabetic females and 23 (35.4%) diabetic females, and in the group of obese females, all the females were diabetic (100%). There was a high statistically significant difference between the three groups regarding the incidence of DM ($P<0.001$).

The results were comparable with Bawah *et al.* [28], who have shown that obese pregnant females are about 109 times likelier to develop GDM than their normal-weight counterparts.

Also, a previous study linked high maternal weight to substantial risk of developing GDM [34].

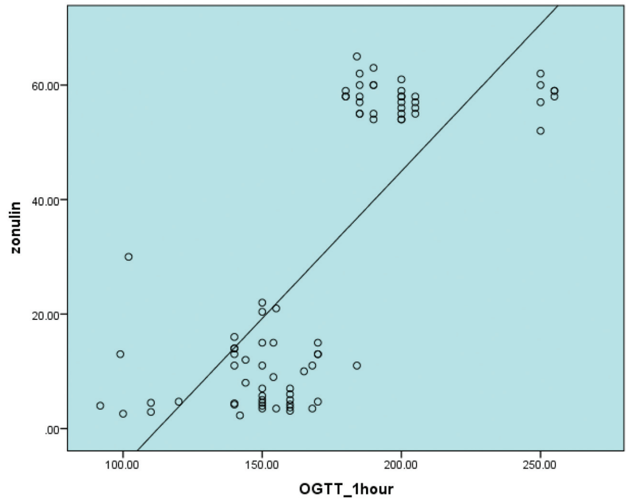
There is a statistically significant positive correlation between serum zonulin levels with BMI, HbA1C, fasting blood glucose, and OGTT values.

Figure 11



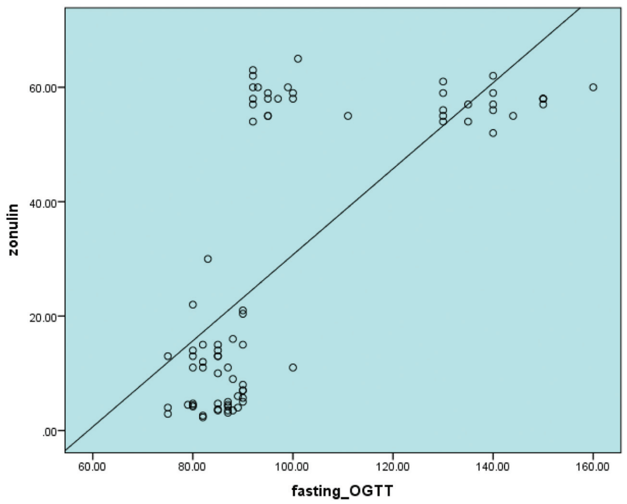
Correlation between serum zonulin levels with FBG. FBG, fasting blood glucose.

Figure 13



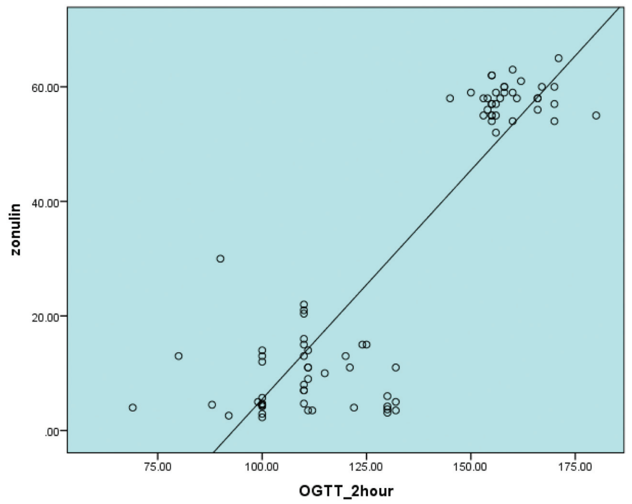
Correlation between serum zonulin levels with OGTT at 1 h. OGTT, oral glucose tolerance test.

Figure 12



Correlation between serum zonulin levels with fasting OGTT. OGTT, oral glucose tolerance test.

Figure 14



Correlation between serum zonulin levels with OGTT at 2 h. OGTT, oral glucose tolerance test.

Table 5 Correlation between body weight according to BMI and diabetes mellitus

DM	Total (*N*=80) [*n* (%)]	Normal weight (*N*=5) [*n* (%)]	Overweight (*N*=65) [*n* (%)]	Obese (*N*=10) [*n* (%)]	*P*
Absent	45 (56.2)	3 (60)	42 (64.6)	0	<0.001*
Present	35 (43.8)	2 (40)	23 (35.4)	10 (100)	

DM, diabetes mellitus. *Serum zinoulin was higher in patients with GDM.

The elevated levels of glucose and insulin may regulate or induce the secretion of zonulin from blood vessels, throughout pregnancy [35].

In this study, the median level of early serum zonulin in the normal-weight females was 5 with range between 2.6 and 63 ng/ml, in the overweight females, was 14 with range between 2.3 and 65 ng/ml, while in the obese females, the median serum zonulin level was 57.5 with range between 55 and 62 ng/ml. The serum level of zonulin was statistically significantly higher in the obese females as compared with normal and overweight females (*P*=0.007).

Plasma level of zonulin was positively associated with BMI. On the other hand, the results in pregnancy were controversial. Houttu *et al.* [36] found that serum levels of zonulin, as a marker of intestinal permeability, were

Table 6 Correlation between serum zonulin and other parameters in the study

	Zonulin	
	r	*P*
BMI	0.535	<0.001*
HbA1C	0.564	<0.001*
FBG	0.560	<0.001*
OGTT fasting	0.704	<0.001*
OGTT 1 h	0.770	<0.001*
OGTT 2 h	0.886	<0.001*

FBG, fasting blood glucose; HbA1C, glycosylated hemoglobin; OGTT, oral glucose tolerance test. *Serum zinoulin was higher in

not significantly different between obese and overweight pregnant females.

Pregnancy is related to significant shifts in the permeability of the intestine with elevated levels of zonulin, which may have a significant responsibility in the noted increases in gestational inflammation, in that way probably playing a role in the GDM development [37].

The results of this study showed that measurement of serum zonulin concentration may be taken as a predictor for an increased risk of GDM, the likely mechanism being induction of inflammation and interference of the action of insulin receptors.

Conclusion

This study revealed that gestational diabetes is a common complication of pregnancy in the middle-aged females, increasing BMI is associated with high incidence of GDM, and serum zonulin level in the first trimester is a sensitive predictor for development of GDM later during pregnancy. The best cut-off point to identify the diabetic females in pregnancy was more than 30 ng/ml with 97.22% sensitivity, 100% specificity, 100% PPV, and 97.8 NPV.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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