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ORIGINAL RESEARCH

Evaluating Serum Zonulin as a Diagnostic Tool for Early Identification of Gestational Diabetes Mellitus

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ABSTRACT

Background: Gestational Diabetes Mellitus (GDM) is a common pregnancy complication with significant maternal and fetal health implications. Early detection and management are critical to reduce the risks associated with GDM, but current diagnostic methods, such as glucose tolerance tests, are invasive and often fail to detect the condition at early stages.This paper explores the potential of serum zonulin as a novel biomarker for the early identification of GDM. **Methods:** This case-control study involved 100 healthy controls and 160 GDM patients .The study was approved by the institutional ethical committee. Serum Zonulin and HOMA IR, OGTT, HBA1C levels were measured, and their correlation was analyzed. **Results:** The present study's findings reveal significant differences in zonulin level between pregnant women with and without Gestational Diabetes Mellitus (GDM). Specifically, women with GDM had significantly higher levels of zonulin (33.26 ± 12.38 ng/ml) compared to women without GDM (13.29 ± 2.01 ng/ml).**Conclusion**: In conclusion, serum zonulin appears to be a promising biomarker for the early prediction of Gestational Diabetes Mellitus (GDM).

Keyword: Gestational Diabetes (GDM), HBA1c, HOMA IR, Serum Zonulin, OGTT, Biomarker.

INTRODUCTION

Gestational diabetes mellitus (GDM) defined as carbohydrate intolerance of variable degree with onset or recognition during pregnancy, has been recently identified as a potential risk factor for Type II Diabetes Mellitus(1). According to the American Diabetes Association criteria, GDM is defined as diabetes diagnosed in the second or third trimester of pregnancy that was not overt prior to gestation(2)

As per International Diabetes Federation (2017) one in seven births is affected by GDM. 16.2% (21.3 million) of live births is to women with hyperglycemia in pregnancy (HIP) (3). India, being home to 69.2 million diabetic subjects, has also become the “diabetes capital of the world” harbouring around four million women with GDM alone (4). Approximately 7% of all pregnancies are complicated by GDM, resulting in more than 200,000 cases annually. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests employed(5).

Zonulin, a protein that plays a key role in regulating intestinal permeability, has emerged as a potential biomarker for various metabolic disorders.Human zonulin (47-kDa protein), also known as prehaptoglobin-2, binds to the epidermal growth factor receptor via protease -activated receptor 2 activation (6). Zonulin is secreted mainly from the liver , but also from enterocytes, adipose tissue, brain, heart, immune cells, lungs, kidney and skin (7,8). Gliadin and bacteria induce zonulin secretion, which increases intestinal permeability, introducing foreign antigens to the immune system and triggering inflammation. Increased zonulin levels are observed in autoimmune diseases associated with TJ dysfunction, including celiac disease (9).

Recent studies suggest that alterations in gut barrier function, as indicated by elevated serum zonulin levels, could be associated with the development of GDM. This study aims to explore the role of serum zonulin as an early predictor of GDM, assessing its potential utility in identifying at-risk pregnancies. Understanding the relationship between serum zonulin

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levels and GDM may offer a new avenue for early detection, improving maternal and fetal outcomes through preventive measures and early interventions.

MATERIAL & METHODOLOGY

The present study comprised a case-control study design involving 160 individuals diagnosed with GDM. Diagnosis was established through clinical history, physical examination, and OGTT and HbA1c%.Cases were selected from the Obs and Gynecology dept of Janana Hospital, Ajmer. Concurrently, age and gender-matched controls (n=100) were selected from the Outpatient Department of the same institution. A comparative analysis of patient data was conducted against the 100 healthy controls.

Patients with GDM who are taking Insulin,smokers, alcoholics, heart disease patients, Hypertensive, endocrine disorders, liver disease and Kidney disease were excluded. Overt Diabetes Type 1 and Type 2 Dm cases are also excluded. Also those who didn't provide consent all were excluded from the study.

Procedure

Blood samples were collected after administrating 50 gm anhydraous Glucose in plain vial or clot activator vial and one on EDTA Vail under aseptic conditions from all the study participants. All samples were centrifuged and analysed after 1 hour. Serum Glucose was estimated using GOD POD methodusing Beckman Coulter Biochemistry Analyzer (DXC700). HbA1C is estimated using HPLC method on D10 biochemical analyzer. Overnight fasting sample was also taken for the estimation of Fasting Serum Insulin and Fasting Glucose on the same day before 1 hour PP Sample. HOMA-IR is calculated using fasting glucose and fasting insulin levels, and it provides a simple, cost-effective way to assess the degree of insulin resistance in a patient.The formula to calculate HOMA-IR is:

$$\text{HOMA-IR} = \frac{\text{Fasting Insulin}(\mu U/mL) \times \text{Fasting Glucose}(mg/dL)}{405}$$

- A value greater than 2.5 is commonly considered indicative of insulin resistance, though this threshold can vary based on population and study.

Data analysis

Collected data were entered into Microsoft Excel spreadsheet and then analysed by IBM SPSS (version 26). Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables.A p-value<0.05was considered significant.

RESULTS

TABLE 1

AGE DISTRIBUTION

Age (in years)	Group 1 Without GDM (n = 100)		Group 2 With GDM (n = 160)		P value
	Number	Percent	Number	Percent	
≤ 30 years	66	66	80	50	p-value = 0.0097
> 30 years	34	34	80	50	
Total	**100**	**100**	**160**	**100**	

The above table shows age distribution in our study. Out of 100 patients in Group 1 (without GDM) 66 patients (66%) were in the age group of < 30 years and 34 patients were in the age group of > 30 years. But in Group 2 (with GDM) out of 160 patients 80 patients were in the age group of < 30 years and 80 patients were in the age group of > 30 years.

The Chi-square (Chi-square = 6.67) reveals a statistically significant association between age and GDM status (p-value = 0.0097). Women with GDM (Group 2) were more likely to be above 30 years of age (50%) compared to women without GDM (Group 1), who were more likely to be below 30 years of age (66%).

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TABLE 2

COMPARISON OF GLYCEMIC MARKERS

Test	Group 1 Without GDM (n = 100)		Group 2 With GDM (n = 160)		P Value
	Mean	±SD	Mean	±SD	
Homeostatic model assessment for insulin resistance (HOMA-IR)	1.123	0.16	3.95	0.8	p<0.0001
Glycated Haemoglobin (HbA1c)	4.9	0.8	6.64	1.17	p<0.0001

The mean HOMA-IR values were significantly higher in Group 2 (With GDM) (3.95 ± 0.8) compared to Group 1 (Without GDM) (1.123 ± 0.16), indicating higher insulin resistance in women with GDM.The mean HbA1c values were also significantly higher in Group 2 (With GDM) (6.64 ± 1.17) compared to Group 1 (Without GDM) (4.9 ± 0.8), indicating poorer glycemic control in women with GDM.

GRAPH 1

COMPARISON OF GLYCEMIC MARKERS

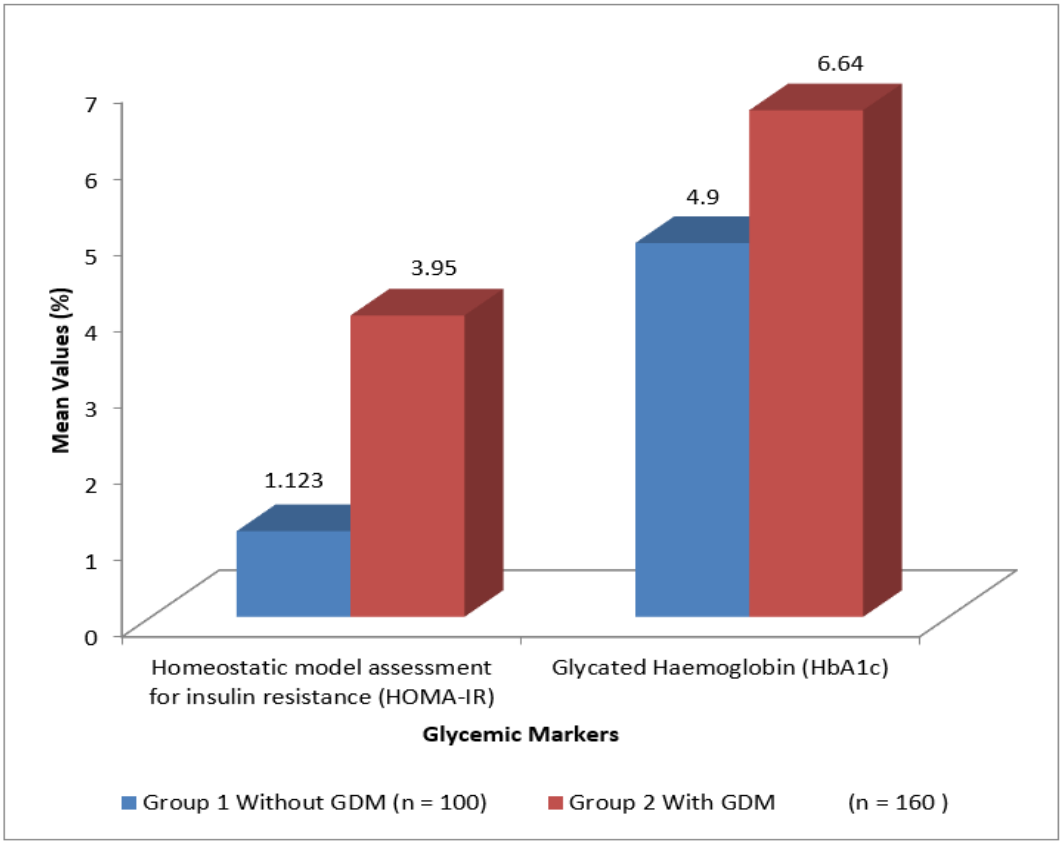

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TABLE 3

COMPARISON OF ZONULIN BETWEEN GROUP 1 & GROUP 2

Parameter	Group 1 Without GDM (n = 100)		Group 2 With GDM (n = 160)		P Value
	Mean	±SD	Mean	±SD	
Zonulin (ng/ml)	13.29	2.01	33.26	12.38	P < 0.0001

The study found highly significant differences (P<0.0001) in zonulin levels between Group 1 (without GDM) and Group 2 (with GDM). The mean zonulin levels were significantly higher in Group 2 (33.26.42 ± 12.38 ng/ml) compared to Group 1 (13.29 ± 2.01 ng/ml.

TABLE 4

CORRELATION OF SERUM ZONULIN LEVELS

WITH GLYCEMIC MARKERS IN CONTROL AND CASE GROUP

Corelation		Group 1 Without GDM (n = 100)	Group 2 With GDM (n = 160)
HOMA- IR	Pearson Correlation	0.0152	0.72
	P Value	0.8807	<0.0001
OGTT	Pearson Correlation	0.152	0.68
	P Value	0.128	<0.0001

In our study Group 1 (without Gestational Diabetes Mellitus, GDM) and Group 2 (with GDM). The analysis correlates HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) and OGTT (Oral Glucose Tolerance Test) values within each group. HOMA-IR and OGTT values show a weak positive correlation (Pearson Correlation = 0.72), but it's statistically significant (P-Value = <0.0001). HOMA-IR and OGTT values show a strong positive correlation (Pearson Correlation = 0.68), which is statistically significant (P-Value < 0.0001).

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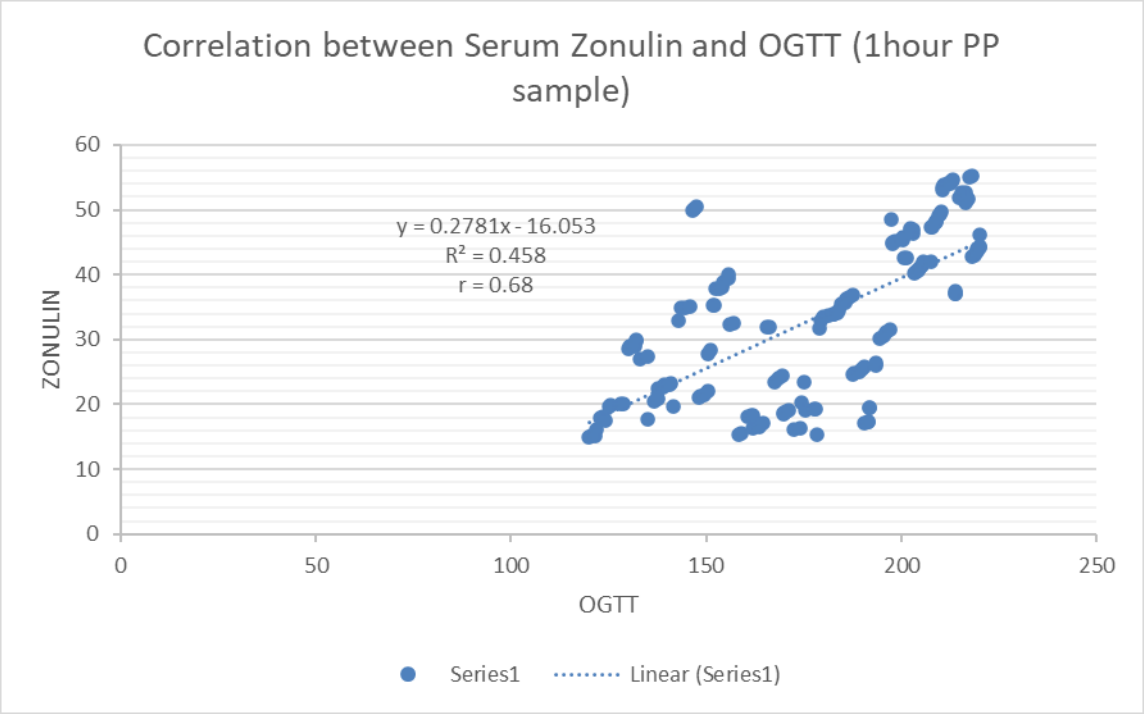
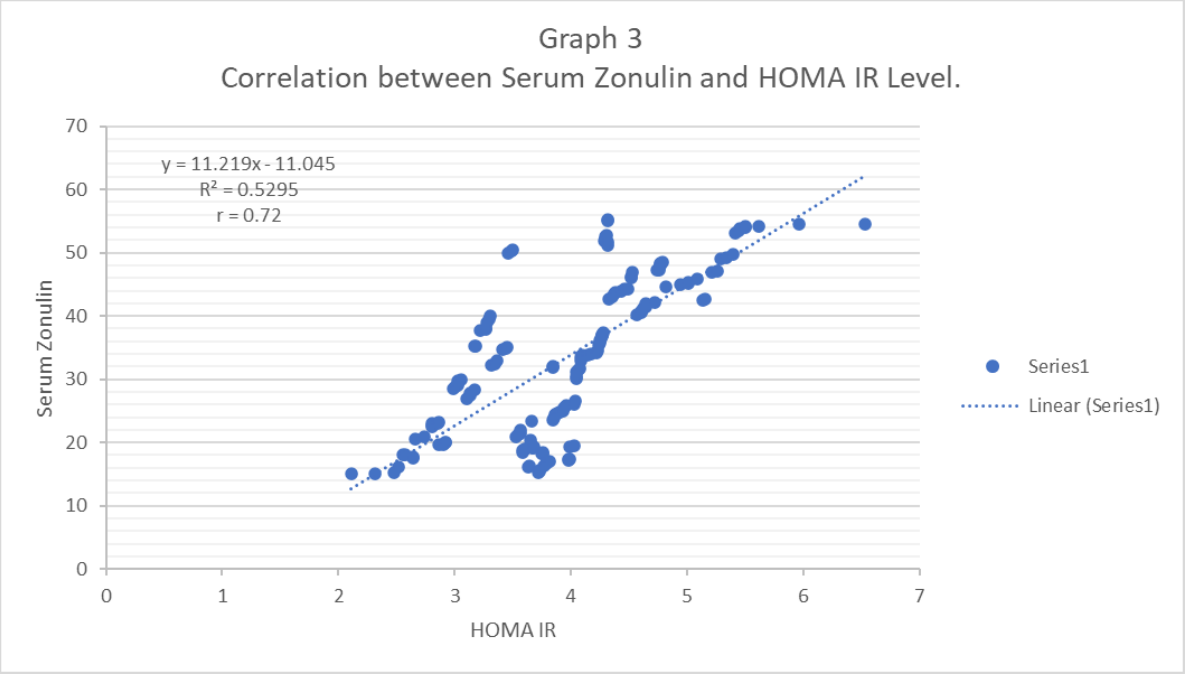


Graph 2: Showing Positive correlation between Serum Zonulin & 1 hour PP Blood sugar level in OGTT .



DISCUSSION

One of the most compelling aspects of this article is its exploration of the biological connection between zonulin and GDM. Zonulin regulates intestinal permeability, and increasing evidence suggests that gut dysfunction and inflammation play a significant role in metabolic diseases, including insulin resistance, which is central to GDM. The article emphasizes that elevated zonulin levels could reflect systemic inflammation and an altered gut barrier, which may contribute to the development of insulin resistance in pregnancy.

The mean HOMA-IR value was significantly higher in women with GDM (3.95 ± 0.8) compared to those without GDM (1.123 ± 0.16), with a p-value of <0.0001.

Furthermore, the study found that women with GDM had significantly higher levels of glycated hemoglobin (HbA1c) compared to women without GDM. The mean HbA1c value was 6.64 ± 1.17% in women with GDM, compared to 4.9 ± 0.8% in women without GDM, with a p-value of <0.0001.

The present study's findings reveal significant differences in zonulin level between pregnant women

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with and without Gestational Diabetes Mellitus (GDM). Specifically, women with GDM had significantly higher levels of zonulin (33.26 ± 12.38 ng/ml) compared to women without GDM (13.29 ± 2.01 ng/ml).This finding aligns with the observations of Ahmed Tijani et al. (2019) reported a significant difference in zonulin levels between GDM cases (59.50 ± 9.0 ng/mL) and non-GDM controls (41.84 ± 7.21 ng/mL)(10). Similarly, Salma et al. (2021) found higher zonulin levels in GDM cases (58 ng/mL) compared to non-GDM controls (7 ng/mL)(11).Demir et al. (2019) and Zehra et al. (2022) also reported higher zonulin levels in GDM cases (32.6 ± 4.8 ng/mL and 28.8 ± 21.9 ng/mL, respectively) compared to non-GDM controls (12.8 ± 3.3 ng/mL and 7.23 ± 11.3 ng/mL, respectively).(12,13)

In conclusion, the majority of the studies suggest that zonulin levels are higher in GDM cases compared to non-GDM controls. However, the differences in zonulin levels between GDM cases and non-GDM controls vary across studies. Further research is needed to understand the relationship between zonulin and GDM.

This concept aligns with the growing body of literature suggesting that metabolic disturbances in pregnancy are not solely a result of increased glucose levels but also involve a complex interplay of immune responses, inflammation, and gut health. By focusing on the role of zonulin, the article highlights a promising area of research that could improve our understanding of GDM beyond the traditional view of it being primarily a glucose-regulation disorder.

CONCLUSION

In conclusion, serum zonulin appears to be a promising biomarker for the early prediction of Gestational Diabetes Mellitus (GDM). The study highlights the potential role of zonulin in regulating intestinal permeability, which may influence insulin resistance and the development of GDM. Elevated serum zonulin levels could serve as an early indicator, allowing for the identification of women at risk for GDM before the onset of significant glucose intolerance. Early detection is crucial for timely intervention, which can reduce the risks of maternal and fetal complications associated with GDM. Further research is needed to confirm these findings and to evaluate the clinical applicability of serum zonulin as a screening tool in routine prenatal care. If validated, serum zonulin could provide a valuable, non-invasive method to predict GDM and improve outcomes for both mothers and their babies.

However, several challenges remain, including the natural fluctuations in zonulin levels during pregnancy, the influence of confounding factors, and the lack of established diagnostic thresholds. While preliminary studies are promising, larger, multicenter studies are essential to validate zonulin as a reliable biomarker for GDM. Furthermore, combining zonulin with other biomarkers of insulin resistance and metabolic dysfunction may enhance diagnostic accuracy and provide a more comprehensive understanding of the pathophysiology of GDM. As research in this area progresses, zonulin could become an integral part of the diagnostic toolkit for GDM, offering a more personalized approach to care and potentially improving long-term health outcomes for both mothers and children.

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