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ORIGINAL ARTICLE

Investigating Obesity-related Biomarkers in Gestational Diabetes Mellitus

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ABSTRACT

Gestational Diabetes Mellitus (GDM) is a significant pregnancy complication characterized by insulin resistance and metabolic dysfunction, with potential adverse outcomes for both mother and child. This study assessed the levels of specific obesity-related biomarkers—Ghrelin, Vaspin, Adiponectin, Neudesin, and Lepocalin—in pregnant women with and without GDM, aiming to elucidate their roles in GDM development and management. Blood samples were collected, and serum biomarker concentrations were analyzed using ELISA. Results revealed significantly elevated levels of Ghrelin (1550.50 ng/mL vs. 1023.62 ng/mL, $p < 0.0001$), Vaspin (1351.98 ng/mL vs. 464.75 ng/mL, $p < 0.0001$), Adiponectin (1121.02 ng/mL vs. 169.92 ng/mL, $p < 0.0001$), Neudesin (1501.43 ng/mL vs. 878.35 ng/mL, $p = 0.003$), and Lepocalin (375.57 ng/mL vs. 115.72 ng/mL, $p < 0.0001$) in GDM cases compared to controls. These biomarkers are associated with energy regulation, insulin sensitivity, inflammation, and appetite control, indicating their potential as early indicators of GDM risk. Notably, IGF-1 levels did not differ significantly between the groups ($p = 0.2110$), suggesting a limited role in GDM pathophysiology. The findings highlight the potential utility of these biomarkers in GDM risk stratification and monitoring. Their elevation, potentially influenced by therapeutic interventions such as metformin, underscores the need for further research to validate their roles and explore personalized therapeutic approaches targeting these biomarkers to improve GDM management and outcomes.

KEY WORDS: Gestational diabetes mellitus, obesity biomarkers, Ghrelin, Vaspin, Adiponectin, Lepocalin, insulin resistance

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common and significant condition affecting many pregnancies worldwide, characterized by impaired glucose tolerance usually arising in the second or third trimester (American Diabetes Association, 2023; ACOG, 2018). This condition not only complicates pregnancy but also poses serious long-term health risks for both mother and child. Maternal complications associated with GDM include an increased risk of hypertensive disorders like preeclampsia, which further complicates labor and delivery, and a higher likelihood of cesarean delivery, often due to macrosomia, or excessive fetal growth (Catalano & Shankar, 2017; Kampmann *et al.*, 2015). For neonates, GDM increases the likelihood of hypoglycemia, respiratory distress syndrome, jaundice, and other metabolic complications (Klein *et al.*, 2020; Buchanan & Xiang, 2005). Long-term, children born to mothers with GDM are at heightened risk for developing obesity, glucose intolerance, and Type 2 diabetes (Al-Goblan *et al.*, 2014; Jang *et al.*, 2017). Additionally, women with a history of GDM are at significantly increased risk of progressing to Type 2 diabetes and metabolic syndrome, highlighting the wide-reaching effects of this condition (Cheng *et al.*, 2016).

The pathophysiology of GDM is multifactorial and involves a convergence of genetic, hormonal, and environmental influences (Fasshauer & Blüher, 2015). A core feature of GDM is insulin resistance, a state where cells become less responsive to insulin, impairing glucose uptake and leading to elevated blood glucose levels (Hivert *et al.*, 2016). During a normal pregnancy, a degree of insulin resistance is expected to facilitate glucose availability to

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the developing fetus. However, in GDM, insulin resistance becomes exaggerated, overwhelming the capacity of pancreatic beta cells to increase insulin production in compensation (Mokkala *et al.*, 2019). This process is compounded by other factors, including chronic inflammation and oxidative stress, both of which disrupt normal glucose metabolism (Hotamisligil, 2006; Ding *et al.*, 2016). Low-grade inflammation, common in obese individuals, further aggravates insulin resistance. Additionally, oxidative stress may damage beta cells, worsening the body's ability to regulate blood glucose effectively (Kirwan *et al.*, 2004; Henson & Castracane, 2006).

Obesity is one of the most significant risk factors for developing GDM. Excess adiposity promotes insulin resistance and disrupts adipose tissue function, which plays a more active role than merely storing fat (Lowe *et al.*, 2020; Santangeli *et al.*, 2018). Adipose tissue secretes a variety of bioactive molecules known as adipokines, which regulate key processes in energy balance, glucose metabolism, inflammation, and insulin sensitivity (Fasshauer *et al.*, 2014). In individuals with obesity, adipokine secretion patterns shift, favoring a pro-inflammatory state that promotes insulin resistance and may contribute to the onset of GDM (Yamamoto *et al.*, 2017). Dysregulated adipokine levels may disrupt normal insulin responses and enhance glucose intolerance, making them potential contributors to the metabolic disturbances observed in GDM patients (Bhattarai *et al.*, 2013).

This study aims to investigate the levels of specific obesity-related biomarkers in pregnant women with and without GDM to better understand the relationship between GDM and adiposity-related metabolic changes (Qiu *et al.*, 2012; Radaelli *et al.*, 2003). The biomarkers examined in this study include Ghrelin, Vaspin, Adiponectin, Insulin-like Growth Factor 1 (IGF-1), Neudesin, and Lipocalin (Chai *et al.*, 2014; Briana & Malamitsi-Puchner, 2009). Each of these biomarkers is involved in various aspects of metabolic regulation, insulin sensitivity, and inflammation. Ghrelin, or the "hunger hormone," regulates appetite and may influence insulin sensitivity (Saxena *et al.*, 2019). Vaspin is an insulin-sensitizing adipokine, while adiponectin has anti-inflammatory properties and enhances insulin sensitivity (Barbour *et al.*, 2007). IGF-1 plays a significant role in cell growth and insulin signaling (Catalano *et al.*, 2007). Neudesin and Lipocalin, though less extensively studied, are implicated in obesity-related metabolic processes and may contribute to GDM pathophysiology (Ferrara, 2007; Knopp *et al.*, 1985).

Through examining these biomarkers, this study seeks to clarify the metabolic and inflammatory changes associated with GDM. Understanding the differences in these biomarkers between GDM and non-GDM pregnancies may help unravel the mechanisms underlying GDM's metabolic dysfunction and support the identification of potential biomarkers for early diagnosis and targeted management strategies. This research aims

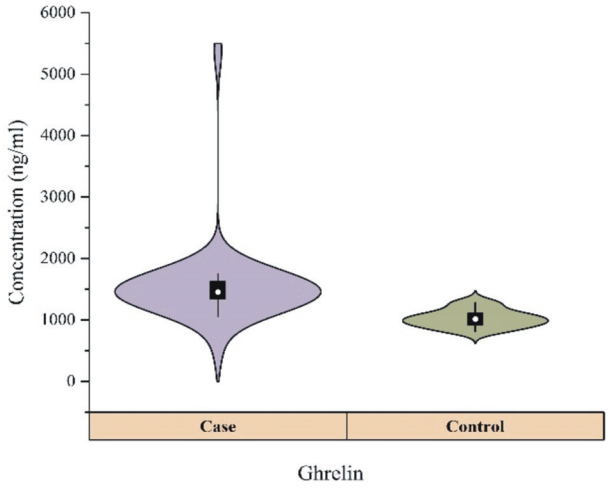


Fig. 1. Elevated Ghrelin levels in GDM cases suggest potential dysregulation in hunger signals and energy metabolism among GDM patients.

p-value < 0.0001 - Significant difference.

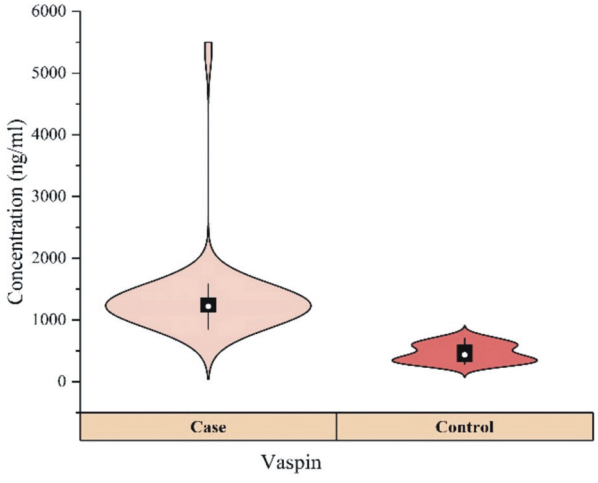


Fig. 2. Significantly higher levels of Vaspin in GDM cases imply its role in insulin resistance, which is a hallmark of GDM.

p-value < 0.0001 - Highly significant difference.

to provide insights into the pathophysiology of GDM and explore the potential for more personalized approaches to improve health outcomes for both mothers and their children (Henson & Castracane, 2006; Fasshauer & Blüher, 2015).

MATERIALS AND METHODS

Study Design and Population

This case-control study was conducted at the Department of Gynaecology, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU), with ethical approval from the institutional review board. Pregnant

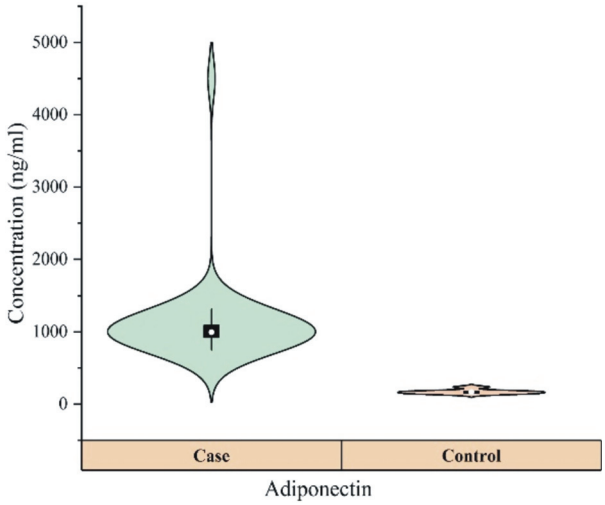


Fig. 3. Elevated levels of Adiponectin in GDM cases may indicate an adaptive response to counteract insulin resistance and inflammation in pregnancy.
p-value < 0.0001 - Strongly significant difference.

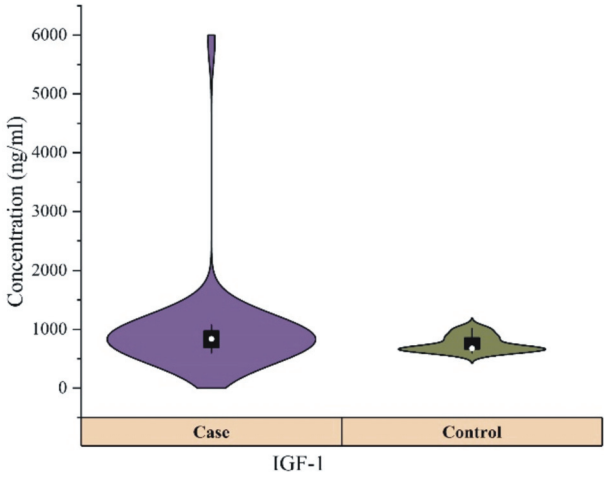


Fig. 4. Similar IGF-1 levels in both groups suggest it may not be a distinguishing marker for GDM in this study.
p-value = 0.2110 - Not statistically significant.

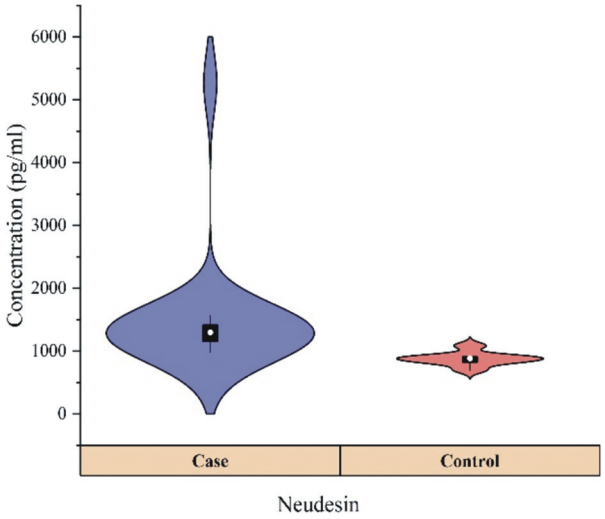


Fig. 5. Higher neudesinlevels in GDM cases may reflect metabolic adjustments or stress responses in GDM.
p-value = 0.0030 - Statistically significant.

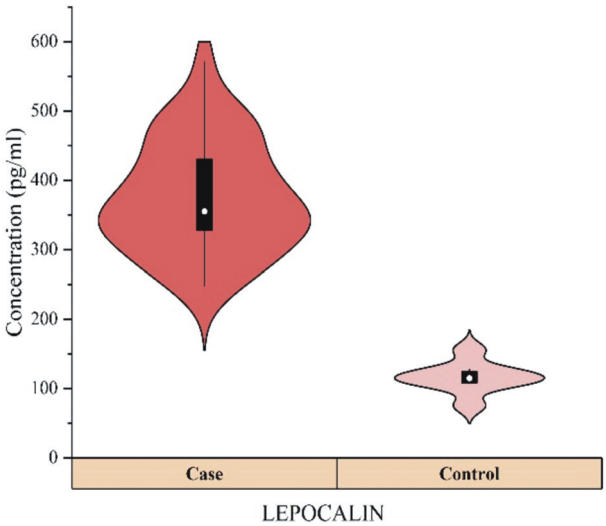


Fig. 6. Significantly elevated Lepocalin in GDM cases suggests it may play a role in inflammation-related insulin resistance in GDM.
p-value < 0.0001 - Highly significant.

women attending antenatal care at IMS-BHU were screened and recruited based on pre-established inclusion and exclusion criteria. A total of 58 pregnant women were enrolled in the study and divided into two groups: 34 diagnosed with GDM and 24 with normal glucose tolerance, serving as controls.

Inclusion Criteria

- Pregnant women aged 18-40 years
- Gestational age between 24 and 28 weeks
- Diagnosis of GDM or abnormal glucose tolerance based on OGTT (Oral Glucose Tolerance Test) results

Exclusion Criteria

- Pre-existing diabetes, hypertension, autoimmune diseases, polycystic ovarian syndrome (PCOS), or metabolic syndrome

Sample Collection and Serum Preparation

Venous blood samples were collected from the antecubital vein of each participant in the fasting state, using standard aseptic techniques. Blood was collected in red-top serum separator tubes and allowed to clot for 30 minutes at room temperature. The samples were then centrifuged at 3000 rpm for 10 minutes to separate serum from cellular components. The serum samples were immediately aliquoted and stored at -80°C until further analysis to ensure biomarker stability.

Biomarker Analysis using ELISA

The serum levels of the selected obesity-related biomarkers–Ghrelin, Vaspin, Adiponectin, IGF-1, Neudesin, and Lipocalin–were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BT-Lab). The procedure for ELISA was carried out as follows:

1. **Plate Preparation:** ELISA plates were coated with capture antibodies specific to each biomarker as per the manufacturer's instructions.
2. **Sample Loading:** Serum samples and standards were diluted and loaded into wells, each sample was analyzed in duplicate to ensure accuracy.
3. **Incubation:** Plates were incubated at room temperature for the recommended time, allowing the biomarkers to bind to the antibodies.
4. **Detection Antibody and Substrate:** After washing the plates to remove unbound material, detection antibodies were added, followed by the enzyme substrate. A colorimetric reaction developed, proportional to the biomarker concentration.
5. **Reading:** Absorbance was measured at 450 nm using the Epoch 2 Microplate Reader (BioTek Instruments), and concentrations were calculated based on the standard curve generated for each biomarker.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 26.0). Descriptive statistics, including means and standard deviations, were calculated for each biomarker in both GDM and control groups. An independent t-test was used to compare mean biomarker levels between the two groups. A p-value of less than 0.05 was considered statistically significant, indicating a meaningful difference in biomarker levels between women with and without GDM.

RESULTS

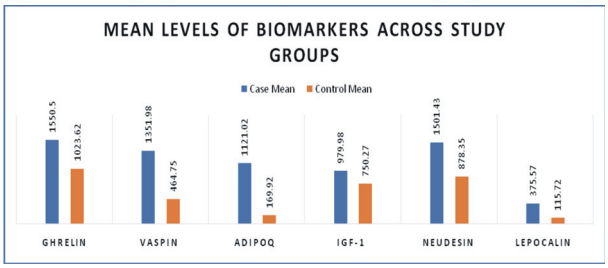
The results of the biomarker analysis in GDM and control groups are presented in Table 1, summarizing the mean ± SD values for each biomarker. The analysis showed significant differences in levels of Ghrelin, Vaspin, Adiponectin, Nesfatin, and Lepocalin between GDM cases and controls. Specifically, GDM cases exhibited elevated levels of Ghrelin, Vaspin, Adiponectin, Nesfatin, and Lepocalin compared to controls, while IGF-1 levels were not significantly different. These elevations suggest that these biomarkers may contribute to the underlying metabolic and inflammatory mechanisms of GDM.

1. **Ghrelin:** Ghrelin levels were notably higher in GDM cases (mean = 1550.50 ng/mL) compared to controls (mean = 1023.62 ng/mL, $p < 0.0001$), as shown in Fig. 1. This elevation could be influenced by metformin use in GDM patients, as metformin has been reported to affect Ghrelin levels. This increase may reflect an adaptive mechanism to regulate energy balance and glucose metabolism, addressing the metabolic dysregulation in GDM.
2. **Vaspin:** The concentration of Vaspin was significantly elevated in the GDM group (mean = 1351.98 ng/mL) compared to the control group (mean = 464.75 ng/mL), with $p < 0.0001$ (Fig. 2). This increase may reflect

Table 1: Comparative Levels of Biomarkers Between Study Groups

Biomarker	Case Mean ± SD (N=34)	Control Mean ± SD (N=24)	p-Value
Ghrelin	1550.50 ± 715.06	1023.62 ± 143.03	<0.0001
Vaspin	1351.98 ± 729.12	464.75 ± 147.58	<0.0001
Adipoq	1121.02 ± 615.81	169.92 ± 30.45	<0.0001
IGF-1	979.98 ± 879.80	750.27 ± 136.30	0.2110
Neudesin	1501.43 ± 978.92	878.35 ± 104.93	0.0030
Lepocalin	375.57 ± 80.06	115.72 ± 21.68	<0.0001

Table 2: Mean Levels of Biomarkers Across Study Groups



a compensatory response to enhance insulin sensitivity in the presence of insulin resistance, which is a key feature of GDM.

3. **Adiponectin:** Adiponectin levels were unexpectedly higher in GDM cases (mean = 1121.02 ng/mL) than in controls (mean = 169.92 ng/mL, $p < 0.0001$), as shown in Fig. 3. This elevation could be attributed to metformin use in GDM patients, as metformin is known to increase Adiponectin levels. This rise may represent an adaptive response to enhance insulin sensitivity, counteracting the heightened insulin resistance observed in GDM.
4. **IGF-1**: IGF-1 levels showed no statistically significant difference between GDM and control groups, with a p-value of 0.2110 (Fig. 4). The lack of significance suggests that IGF-1 may not be directly involved in the metabolic disturbances linked to GDM in this population.
5. **Neudesin:** Neudesin levels were significantly elevated in the GDM group (mean = 1501.43 ng/mL) compared to controls (mean = 878.35 ng/mL), with a p-value of 0.0030. This suggests that neudesin may play a role in the metabolic stress and inflammatory response associated with gestational diabetes mellitus.
6. **Lepocalin:** Lepocalin levels were significantly higher in GDM cases (mean = 375.57 ng/mL) than in the control group (mean = 115.72 ng/mL, $p < 0.0001$) (Fig. 5). This biomarker's association with inflammation may indicate its involvement in the pro-inflammatory state observed in GDM.

Non-Significant Biomarker: **IGF-1**

IGF-1 levels showed no statistically significant difference between GDM and control groups, with a p-value of 0.2110 (Fig. 4). The lack of significance suggests that IGF-1 may not be directly involved in the metabolic disturbances linked to GDM in this population.

Summary of Findings

Overall, the study demonstrates that Ghrelin, Vaspin, Adiponectin, Nesfatin, and Lepocalin are significantly elevated in GDM cases, pointing to their potential roles in GDM's pathophysiology and as markers for early detection. The absence of a significant change in IGF-1 levels suggests that its role in GDM-specific metabolic alterations may be limited in this cohort.

DISCUSSION

This study's findings highlight substantial differences in obesity-related biomarkers between pregnant women with GDM and those without, demonstrating complex relationships among obesity, insulin resistance, and metabolic dysregulation in GDM. This section situates our findings within the current literature, examining the potential implications of these biomarkers in the pathogenesis of GDM and their clinical use.

Ghrelin: Ghrelin, commonly referred to as the "hunger hormone," is primarily synthesized in the stomach and plays a crucial role in appetite regulation and energy homeostasis. In this study, we observed significantly elevated ghrelin levels in GDM patients compared to controls, supporting its potential involvement in metabolic imbalance in GDM. This elevation may reflect a compensatory mechanism in response to increased insulin resistance, as the body attempts to maintain energy equilibrium by augmenting ghrelin secretion.

Previous studies have similarly reported elevated ghrelin levels in association with insulin resistance and increased caloric intake, which are pivotal in the metabolic disruptions seen in GDM. However, conflicting evidence exists, with some studies reporting reduced ghrelin levels in GDM, highlighting the complex role of this biomarker in pregnancy-related metabolic alterations (H.S. Brink *et al.*, 2019).

Our findings align with recent research by Doogue *et al.* (2009), which suggests that ghrelin levels are significantly influenced by metformin treatment in GDM patients. In our cohort, patients were on metformin therapy, which is known to regulate energy metabolism and improve insulin sensitivity. The increased ghrelin levels observed may be attributed to metformin's effects on hormonal regulation, indicating a need for further investigation into the biomarker's response to therapeutic interventions.

Vaspin: Vaspin, a serine protease inhibitor predominantly secreted by visceral adipose tissue, has shown insulin-sensitizing properties, making it a promising biomarker for insulin resistance-related conditions. In our study, vaspin levels were markedly elevated in GDM cases compared to controls, consistent with prior findings in obesity and type 2 diabetes (Jang *et al.*, 2017; Bhattarai *et al.*, 2013).

Elevated vaspin levels may represent a compensatory mechanism to counteract insulin resistance, a hallmark of

GDM. Vaspin is believed to inhibit inflammatory processes and improve insulin sensitivity, thereby playing a protective role in GDM (Chai *et al.*, 2014; Fasshauer *et al.*, 2014). Studies have also linked increased vaspin levels to the body's efforts to regulate glucose metabolism under metabolic stress conditions, further supporting its therapeutic potential (Santangeli *et al.*, 2018; Radaelli *et al.*, 2003).

The findings in this study align with the notion that vaspin serves as an adaptive response to the persistent insulin resistance observed in GDM, highlighting its potential as a biomarker for disease severity and therapeutic monitoring.

Adiponectin : Adiponectin, known for its anti-inflammatory and insulin-sensitizing properties, is typically inversely related to body fat, with lower levels observed in obesity and related metabolic disorders. However, in this study, we observed significantly elevated adiponectin levels in GDM patients compared to controls.

Brittany *et al.* (2023) proposed that during pregnancy and GDM, adiponectin plays a crucial role in regulating energy metabolism and insulin sensitivity. Lower adiponectin levels have been widely regarded as a marker of GDM in several populations, consistent with its expected relationship with insulin resistance. However, the upregulation of adiponectin observed in our study may reflect the influence of metformin therapy.

Dan Zhao *et al.* (2023) reported that metformin, commonly used for GDM management, can modulate adipokine profiles and improve insulin sensitivity. In our cohort, the increased adiponectin levels might be attributed to metformin's regulatory effects, suggesting that the drug could influence biomarker levels through its glucose-lowering and anti-inflammatory properties. These findings emphasize the importance of considering treatment effects when interpreting biomarker variations in GDM.

Insulin-like Growth Factor 1 (IGF-1): IGF-1 is a hormone known for its growth-promoting and insulin-mimetic properties, playing an essential role in embryonic development and glucose homeostasis. In this study, no significant difference in IGF-1 levels was observed between GDM and control groups, suggesting that IGF-1 may not serve as a primary contributor to GDM-specific metabolic alterations.

Research on IGF-1 levels in GDM has yielded conflicting results. While some studies have reported elevated IGF-1 levels due to altered maternal and placental growth signaling, others, including our findings, have found no significant variations (Catalano *et al.*, 2007; Knopp *et al.*, 1985). This variability suggests that IGF-1's role in GDM may depend on other factors such as maternal obesity or interactions with adipokines. Therefore, IGF-1 likely plays an ancillary role in GDM pathophysiology rather than being a direct marker of metabolic dysfunction.

Neudesin: Neudesin, a neurotrophic factor involved in energy metabolism and cell survival, was found to be significantly elevated in GDM patients in this study. The increased levels of Neudesin may reflect a metabolic stress response aimed at addressing inflammation and maintaining cellular viability during pregnancy.

Research on Neudesin in GDM is limited; however, its involvement in energy regulation and neuroprotection highlights its potential as a biomarker. Elevated Neudesin levels in GDM cases may indicate the body's adaptive mechanisms to counteract metabolic stress and inflammation associated with the condition (Barbour *et al.*, 2007; Zhao *et al.*, 2023). These findings underscore the need for further research into the role of Neudesin in pregnancy-related metabolic adjustments.

Lepocalin: Lepocalin, a protein linked to immune response, oxidative stress, and inflammation, was markedly elevated in GDM patients in this study. The significant increase in Lepocalin levels suggests its involvement in the pro-inflammatory state commonly observed in GDM.

Studies have shown that Lepocalin is associated with heightened oxidative stress and inflammation in obesity-related conditions, including GDM (Fasshauer *et al.*, 2014; Radaelli *et al.*, 2003). This biomarker's role in modulating inflammatory pathways positions it as a potential candidate for evaluating inflammatory and oxidative stress responses in GDM. Its consistent elevation in GDM patients highlights its utility as a marker for disease severity and progression.

Examination in Context of Previous Studies

Our findings align with much of the prevailing literature on obesity biomarkers in GDM, while also revealing unique inconsistencies that highlight the intricate interplay of biomarkers during pregnancy. For instance, the observed increases in ghrelin and vaspin levels corroborate prior studies, which emphasize their roles in energy metabolism and compensatory mechanisms to address insulin resistance in GDM patients (Jang *et al.*, 2017; Fasshauer *et al.*, 2014).

However, the unexpected elevation of adiponectin in GDM cases observed in this study contrasts with its typically lower levels in non-pregnant individuals with obesity and insulin resistance. This divergence underscores the distinct metabolic milieu during pregnancy, where hormonal and physiological changes may uniquely influence adipokine secretion (Brittany *et al.*, 2023). Additionally, the influence of metformin, widely used for managing GDM, on adiponectin and ghrelin levels

further complicates the interpretation of these results, necessitating further research.

The elevation of neudesin and lepocalin also provides insights into the inflammatory and neuroprotective responses in GDM, aligning with their suggested roles in managing metabolic stress during pregnancy (Barbour *et al.*, 2007; Zhao *et al.*, 2023). The absence of significant changes in IGF-1 levels suggests that its role in GDM may be more peripheral, influenced by factors like maternal obesity rather than directly contributing to the disease's pathophysiology (Catalano *et al.*, 2007). Together, these findings emphasize the need for additional studies to unravel the complexities of biomarker dynamics during pregnancy.

ClinicalConsiderations

Understanding the biomarker profiles associated with GDM offers significant clinical potential for improving early detection, treatment customization, and patient outcomes. Biomarkers such as ghrelin, vaspin, neudesin, and lepocalin may serve as early indicators of metabolic dysregulation, helping clinicians identify at-risk individuals during pregnancy. The observed increases in these biomarkers in GDM cases point to their utility in diagnosis and monitoring, particularly in high-risk populations like obese women.

For example, ghrelin and vaspin levels could be integrated into predictive models for GDM risk stratification, while tracking adiponectin levels may provide insights into the effects of interventions like metformin. Similarly, the elevation of inflammatory markers like lepocalin and neuroprotective factors like neudesin highlights their potential as tools for assessing disease severity and progression.

The absence of significant changes in IGF-1 levels, despite its known roles in glucose metabolism and fetal development, indicates that not all biomarkers are equally affected by GDM. This finding highlights the importance of a multi-biomarker approach to better capture the complexity of GDM's pathophysiology. Moving forward, incorporating biomarker profiling into routine clinical practice could enable more personalized care, improving outcomes for both mothers and infants.

CONCLUSION

This study underscores notable differences in key obesity-related biomarkers, including ghrelin, vaspin, adiponectin, and lepocalin, between pregnant women with GDM and those without. These findings reveal a complex interplay of metabolic, inflammatory, and regulatory factors that contribute to the pathophysiology of GDM.

The observed elevations in ghrelin, vaspin, and lepocalin suggest compensatory mechanisms aimed at regulating insulin sensitivity, energy balance, and inflammation in response to heightened metabolic stress. The unexpected upregulation of adiponectin in GDM cases highlights unique pregnancy-specific adaptations, potentially influenced by metformin treatment, that may mitigate some aspects of insulin resistance.

Although IGF-1 levels showed no significant differences, its neutral role suggests that not all growth-related variables are equally impacted by the metabolic disruptions associated with GDM. The findings from this study suggest that these biomarkers have the potential to act as early indicators for GDM diagnosis and management, helping clinicians tailor treatment strategies to individual metabolic profiles.

Future research should focus on larger cohorts and longitudinal studies to validate these results and clarify the roles of these biomarkers in GDM. Such efforts will enhance the integration of biomarker profiling into clinical practice, improving the early detection, monitoring, and personalized management of GDM to mitigate its long-term consequences for both mothers and their children.

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