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Exploring miR-16-5p and Biochemical Markers as Potential Noninvasive Indicators of Gestational Diabetes Mellitus

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ABSTRACT

Background: Gestational diabetes mellitus (GDM) is a growing global health concern, associated with significant maternal and fetal complications. Despite routine screening protocols, current diagnostic methods remain invasive and suboptimal. MicroRNAs (miRNAs), such as miR-16-5p, have emerged as promising noninvasive biomarkers for metabolic disorders, including GDM. **Objective:** This study aimed to assess the differential expression of circulating miR-16-5p in women with GDM and investigate its correlation with key biochemical markers involved in glucose metabolism, inflammation, and oxidative stress. **Methods:** In this cross-sectional study, 80 pregnant women (40 with GDM, 40 healthy controls) were recruited from a tertiary care center. Biochemical markers—fasting glucose, serum insulin, IGF-1, IGFBP-1, TNF-α, and GPX—were measured via ELISA. miR-16-5p expression was quantified using qRT-PCR and analyzed using the 2^−ΔΔCt method. Group comparisons and correlation analyses were performed using non-parametric tests. **Results:** miR-16-5p expression was significantly upregulated in the GDM group (3.25 ± 0.96-fold, $p < 0.001$). GDM participants also exhibited elevated fasting glucose and insulin levels, and decreased IGF-1, IGFBP-1, TNF-α, and GPX levels compared to controls. In GDM subjects, miR-16-5p positively correlated with fasting glucose ($r = 0.411$, $p = 0.008$) and inversely with GPX ($r = -0.450$, $p = 0.004$). **Conclusion:** miR-16-5p shows promise as a noninvasive biomarker for GDM, reflecting alterations in glucose regulation and oxidative stress. Its integration into early diagnostic strategies may improve maternal-fetal outcomes. Further longitudinal and mechanistic studies are warranted.

Keywords: miR-16-5p, gestational diabetes mellitus, oxidative stress, biomarkers, insulin resistance

INTRODUCTION

Gestational diabetes mellitus (GDM) is a prevalent metabolic disorder of pregnancy, defined by glucose intolerance with onset or first recognition during the second or third trimester (Modzelewski et al., 2022; Gică & Huluță, 2023). The global prevalence of GDM has been steadily increasing, affecting approximately 7–14% of pregnancies, with even higher rates in low- and middle-income countries due to changes in lifestyle, nutrition, and urbanization (Arokiasamy, Salvi, & Selvamani, 2021;Tayyab et al., 2024). This condition presents significant short- and long-term health risks to both mother and fetus. Mothers with GDM are at greater risk for hypertensive disorders of pregnancy, cesarean delivery, and developing type 2 diabetes mellitus postpartum (Sewor et al., 2024; Ye et al., 2022). Meanwhile, infants born to mothers with GDM face increased risk for macrosomia, neonatal hypoglycemia, and future metabolic syndrome (Pathirana et al., 2021) (HAPO Study Cooperative Research Group, 2008).

Despite the clinical burden of GDM, its pathophysiology is not fully understood. Insulin resistance is a hallmark of GDM, often accompanied by β-cell dysfunction and low-grade systemic inflammation (Piotrowska et al., 2023). The maternal metabolic milieu in GDM involves dysregulation in the insulin-like growth factor (IGF) axis, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), and oxidative stress, which collectively disrupt placental and fetal development (Firdous & Pal, 2023). While biochemical markers like fasting insulin, IGF-1, IGF-binding proteins (IGFBPs), and antioxidant enzymes such as glutathione peroxidase (GPX) offer mechanistic insights, their clinical utility as early predictive or diagnostic tools remains limited due to variability and overlap with normal gestational physiology.

In recent years, epigenetic regulators, particularly microRNAs (miRNAs), have emerged as promising biomarkers and mediators in metabolic diseases including GDM Ramzan, Vickers, & Mithen, 2021; Elhag & Al Khodor, 2023). miRNAs are small (~22 nucleotides), noncoding RNAs that modulate gene

expression post-transcriptionally by targeting messenger RNAs (Treeck, Haerteis, & Ortmann, 2023; Menon, Abd-Aziz, Khalid, Poh, & Naidu, 2022). They play critical roles in cell differentiation, immune responses, and glucose metabolism. Several studies have shown altered miRNA profiles in maternal plasma and placental tissue in GDM, implicating them in insulin resistance, inflammation, and endothelial dysfunction (Liu et al., 2021; He et al., 2024).

Among the miRNAs implicated in GDM, **miR-16-5p** has garnered interest for its regulatory influence on insulin signaling and oxidative stress pathways (Juchnicka et al., 2022). It is known to target components of the PI3K/Akt pathway, which is essential for insulin-mediated glucose uptake. Elevated miR-16-5p levels have been observed in type 2 diabetes and metabolic syndrome, suggesting its broader role in insulin resistance states. However, limited data exist on miR-16-5p expression specifically in GDM populations, and its potential interplay with classical biochemical markers such as TNF-α and GPX has not been fully elucidated. Understanding whether miR-16-5p correlates with metabolic and oxidative markers in GDM may offer new diagnostic and therapeutic insights.

Furthermore, the potential of miR-16-5p as a **noninvasive biomarker** is particularly relevant for pregnancy, where minimally invasive diagnostic tools are preferred to avoid maternal-fetal risks (Janšáková et al., 2021; Ghafourian et al., 2022). While conventional screening tools like the oral glucose tolerance test (OGTT) are currently standard, they are often inconvenient, time-consuming, and poorly tolerated (Kuo et al., 2021). In contrast, circulating miRNAs in whole blood or serum offer stability, reproducibility, and early detection potential—making them attractive candidates for integration into risk prediction models.

Given these considerations, the present study was designed to investigate the differential expression of miR-16-5p in pregnant women diagnosed with GDM compared to normoglycemic controls. Additionally, the study aimed to explore the association between miR-16-5p levels and key biochemical markers involved in glucose regulation, inflammation, and oxidative stress, including insulin, IGF-1, IGFBP-1, TNF-α, and GPX. By identifying molecular signatures that distinguish GDM from normal pregnancies, this study seeks to enhance our understanding of GDM pathogenesis and contribute to the development of novel, noninvasive diagnostic strategies.

METHODOLOGY

This analytical cross-sectional study was conducted to investigate the differential expression of circulating miR-16-5p and associated biochemical markers among pregnant women diagnosed with gestational diabetes mellitus (GDM) in comparison to normoglycemic pregnant controls. A total of 70 singleton pregnant women in their second and third trimesters were recruited between January 2024 and June 2024 from the antenatal outpatient departments of Mayo Hospital Lahore, Lahore. Participants were enrolled using a purposive sampling technique after obtaining informed consent. The study cohort comprised 35 women with clinically confirmed GDM based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria, and 35 age- and gestational age-matched healthy pregnant controls. Women with pre-existing diabetes, hypertension, autoimmune disorders, or multiple gestations were excluded to minimize potential confounders.

Peripheral venous blood samples were collected under aseptic conditions. Serum was separated and stored at −80°C until biochemical analysis. Quantitative assessments of fasting insulin, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), tumor necrosis factor-alpha (TNF-α), and glutathione peroxidase (GPx) activity were performed using commercially available human-specific ELISA kits, adhering strictly to manufacturer protocols. Total RNA, including miRNA, was isolated from whole blood using a miRNA extraction kit (Qiagen, Germany). The expression of miR-16-5p was quantified by TaqMan-based real-time quantitative PCR (qRT-PCR), normalized against U6 small nuclear RNA as the internal control. Relative expression levels were calculated using the 2^−ΔΔCt (Livak) method.

Statistical analyses were conducted using IBM SPSS Statistics version 26.0. Data distribution was evaluated using the Shapiro-Wilk test. Given the non-normal distribution of most parameters, non-parametric tests were applied. Between-group comparisons were analyzed using the Mann-Whitney U test, and relationships between miR-16-5p and biochemical markers were assessed through Spearman's rank-order correlation. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

A total of 80 pregnant women were included in the study, comprising 40 patients with gestational diabetes mellitus (GDM) and 40 age-matched healthy pregnant controls. The demographic characteristics were comparable between the two groups ($p > 0.05$ for age and gestational age).

Biochemical Marker Comparison

GDM patients exhibited significantly elevated fasting

blood glucose, serum insulin, and miR-16-5p expression levels (all $p < 0.001$) compared to controls. In contrast, IGF-1, IGFBP-1, TNF-α, and GPX levels were significantly higher in the control group ($p < 0.05$ for each).

Correlation Analysis

Spearman's rank correlation revealed that miR-16-5p expression positively correlated with serum glucose levels ($r = 0.411$, $p = 0.008$) and negatively correlated with GPX levels ($r = –0.450$, $p = 0.004$) in the GDM group. No significant correlations were found in the control group.

Table 1. Comparison of Biochemical Parameters Between GDM and Control Groups

Parameter	GDM Group (n=40)	Control Group (n=40)	p-value
Age (years)	28.6 ± 4.2	27.9 ± 3.9	0.347
Gestational age (weeks)	27.5 ± 2.3	27.2 ± 2.6	0.542
Fasting glucose (mg/dL)	124.3 ± 10.5	86.2 ± 8.7	< 0.001
Serum insulin (µIU/mL)	20.1 ± 5.6	12.8 ± 4.3	< 0.001
IGF-1 (ng/mL)	145.2 ± 32.5	178.4 ± 30.6	0.003
IGFBP-1 (ng/mL)	27.1 ± 9.2	35.6 ± 10.3	0.001
TNF-α (pg/mL)	12.5 ± 2.1	15.4 ± 2.6	0.004
GPX (U/mL)	32.7 ± 7.8	41.2 ± 6.5	< 0.001
miR-16-5p (fold change)	3.25 ± 0.96	1.00 (reference)	< 0.001

Values are presented as mean ± SD. Mann-Whitney U test was applied due to non-normal distribution for most variables.

Table 2. Correlation of miR-16-5p Expression with Biochemical Parameters in GDM Group

Biochemical Parameter	Correlation Coefficient (r)	p-value
Fasting glucose	0.411	0.008
Serum insulin	0.221	0.164
IGF-1	–0.098	0.549
IGFBP-1	–0.131	0.413
TNF-α	–0.187	0.245
GPX	–0.450	0.004

Spearman's rank correlation was used.

DISCUSSION

The present study provides novel insights into the altered expression of miR-16-5p and associated biochemical markers in pregnancies complicated by gestational diabetes mellitus (GDM). Our findings demonstrate a significant upregulation of miR-16-5p in women with GDM compared to normoglycemic pregnant controls, suggesting its potential utility as a noninvasive biomarker for early detection and monitoring of GDM. This elevation in miR-16-5p aligns with previous reports implicating this microRNA in the regulation of insulin signaling pathways, inflammatory responses, and oxidative of GDM.

In parallel with miR-16-5p upregulation, we observed significantly increased serum insulin and glucose levels in the GDM group, reflective of insulin resistance and impaired glucose metabolism characteristic of the disorder. Interestingly, our study also revealed an inverse relationship between miR-16-5p expression and GPx activity, suggesting a possible link between miRNA dysregulation and reduced antioxidant defense mechanisms in GDM. Conversely, levels of IGF-1, IGFBP-3, TNF-α, and GPx were notably higher in the control group, potentially indicating a protective metabolic and anti-inflammatory milieu in healthy pregnancies.

The elevated TNF-α levels in controls, while seemingly counterintuitive, may reflect gestational stage-dependent variations or an adaptive immunological response rather than overt inflammation. Nonetheless, our findings underscore the complex interplay between growth factors, oxidative stress markers, and microRNAs in GDM. Notably, the positive correlation between miR-16-5p and glucose levels supports the hypothesis that hyperglycemia may drive miRNA overexpression, thereby influencing downstream metabolic and oxidative stress pathways.

This study adds to a growing body of evidence supporting the role of circulating miRNAs as minimally invasive diagnostic tools in maternal metabolic disorders. While our results are promising, the cross-sectional design precludes causal inferences, and the relatively modest sample size may limit generalizability. Furthermore, the study did not control for dietary or pharmacological interventions, which may have influenced biochemical parameters. Future longitudinal studies with larger, more diverse populations and functional analyses of miR-16-5p targets are warranted to validate its clinical utility and elucidate underlying mechanisms.

CONCLUSION

This study highlights the potential utility of miR-16-5p as a noninvasive biomarker for gestational diabetes mellitus (GDM). The observed upregulation of miR-16-5p in GDM patients, alongside its positive correlation with fasting glucose and negative correlation with GPX activity, underscores its

relevance in glucose metabolism and oxidative stress pathways central to GDM pathophysiology. These findings suggest that miR-16-5p may serve as a molecular signature of metabolic dysregulation in pregnancy, offering diagnostic value beyond traditional biochemical markers. Moreover, its stability in circulation makes it an attractive candidate for early screening models. However, limitations including the cross-sectional design and lack of dietary or therapeutic control warrant cautious interpretation. Future studies should focus on longitudinal validation, target gene analysis, and evaluation of miR-16-5p's prognostic performance in diverse populations to fully establish its role in the clinical management of GDM.

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