

Molecular biomarkers for gestational diabetes mellitus and postpartum diabetes

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Abstract

Gestational diabetes mellitus (GDM) is a growing public health problem worldwide that threatens both maternal and fetal health. Identifying individuals at high risk for GDM and diabetes after GDM is particularly useful for early intervention and prevention of disease progression. In the last decades, a number of studies have used metabolomics, genomics, and proteomic approaches to investigate associations between biomolecules and GDM progression. These studies clearly demonstrate that various biomarkers reflect pathological changes in GDM. The established markers have potential use as screening and diagnostic tools in GDM and in postpartum diabetes research. In the present review, we summarize recent studies of metabolites, single-nucleotide polymorphisms, microRNAs, and proteins associated with GDM and its transition to postpartum diabetes, with a focus on their predictive value in screening and diagnosis.

Keywords: Gestational diabetes mellitus; Biomarkers; Metabolomics; Proteomics; microRNA; Single-nucleotide polymorphism

Introduction

Gestational diabetes mellitus (GDM), one of the most common pregnancy complications, is defined as diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes prior to gestation.^[1] The prevalence of GDM varies widely depending on population characteristics and diagnostic criteria. In 2017, the International Diabetes Federation reported that around 21.3 million live births (16.2%) worldwide were affected by hyperglycemia in pregnancy, including 18.4 million cases involving GDM. Notably, estimates of the raw prevalence of hyperglycemia in pregnancy range from 9.5% in Africa to 26.6% in Southeast Asia.^[2] Risk factors for GDM include pre-pregnancy body mass index (BMI), advanced maternal age, ethnicity, family history of diabetes, smoking, and perfluorochemicals during pregnancy.^[3-5] GDM is a cause of morbidity and mortality in both mothers and infants. Patients with GDM have a high risk of pre-eclampsia, polyhydramnios, operative delivery, and birth canal lacerations, while short-term consequences for offspring include shoulder dystocia, macrosomia, neonatal hypoglycemia, jaundice, and perinatal mortality. While GDM usually resolves following delivery, it can

have a long-term impact on both the mother and the infant, including increased risks of hyperglycemia, diabetes, obesity, and cardiovascular diseases in the future. This contributes to the transgenerational cycle of diabetes and cardiometabolic disorders.^[6,7] Therefore, GDM is a considerable threat to the health of mothers and infants worldwide, and research on its pathogenesis, early diagnosis, and intervention is necessary.

Similar to diabetes, GDM development has multiple mechanisms, including β -cell dysfunction, insulin resistance, adipose tissue dysfunction, gluconeogenesis, gut microbiota dysbiosis, and oxidative stress.^[8] Owing to this complexity, our current understanding of the pathogenesis of this disease remains limited.

Biomarkers are quantifiable indicators of the physiological and pathological status of an organism. They are clinically useful because they can be used to assess the risk of disease development in healthy individuals. In the subclinical phase of diseases, biomarkers serve as screening tools for diagnosis, the prevention of progression, and monitoring pharmacological responses to therapeutic interventions.^[9] Many molecular biomarkers for GDM have been investigated, including metabolites, single-nucleotide

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polymorphisms (SNPs), microRNAs (miRNAs), and proteins. These biomarkers may complement existing clinical risk factors to identify women at high risk of developing GDM during pregnancy as well as type 2 diabetes mellitus (T2D) after delivery. Furthermore, they provide insights into the pathophysiology of the disease and reveal the molecular mechanisms underlying the development of GDM.^[10-12] Hence, molecular biomarkers have contributed substantially to GDM research in recent decades.

Metabolomics is the comprehensive analysis of low-molecular-weight compounds, known as metabolites, in biofluids, cells, and tissues. As the end products of metabolic processes, metabolites can reflect the internal physiological status of an organism, gene expression, and changes in the response to environmental factors. Due to the high sensitivity, even subtle changes in metabolic networks can be detected. Moreover, metabolomics provides an integrated profile of the biological status, without considering the effects of individual factors.^[13] The most frequently used experimental technologies in metabolomics are liquid or gas chromatography–mass spectrometry (MS) and proton nuclear magnetic resonance (¹H NMR) spectroscopy. MS-based approaches provide high throughput, sensitivity, and versatility, while NMR-based methods offer an overview of structural information, dynamic process, and higher reproducibility.^[14,15] Though MS is usually more sensitive than ¹H NMR, the biological fluid in NMR does not require any physical or chemical treatment prior to the analysis, when compared with MS-based methods. Furthermore, NMR does not damage analytes, which is useful when exploring metabolites in tissues that should be used in further experiments.^[13] Untargeted and targeted profiling are the main methodologies used for metabolomics. Untargeted metabolomics allows for studies of metabolites without *a priori* information and is therefore suitable for candidate biomarker discovery, hypothesis generation, and analyses of metabolic mechanisms. In contrast, targeted metabolomics is usually employed for the analysis of specific metabolites. This type of analysis can be used to validate biomarkers and study metabolic pathways of interest.^[16] Since samples of biological fluids, such as blood or urine, can be collected fairly easily, metabolites and their biological responses to diseases can be easily studied in detail. Metabolic groups include branched-chain amino acids (BCAAs), aromatic amino acids, sulfur-containing amino acids, phospholipids, and other metabolites, which mainly participate in amino acid, lipid, and carbohydrate metabolism.^[17-19]

SNPs refer to alterations in a single nucleotide in the genome sequence. While most SNPs do not alter the function or expression of genes, some influence gene expression and contribute to diseases. Comparisons of SNP frequencies between individuals with a disease and control individuals can reveal candidate loci associated with the disease.^[20] One general approach for these studies is the candidate gene approach, which is used to investigate the association between a particular susceptibility gene and a phenotype.^[21] Technologies such as TaqMan assay, SNaPshot, and PCR-restriction fragment

length polymorphism methods are widely used.^[22] For the reason that each SNP contains limited genetic information, a great deal of effort has been devoted to improving the throughput of genotyping. SNPlex, the Illumina BeadArray, the Sequenom MassARRAY iPLEX platform, and other SNP genotyping platforms are developed to serve different purposes.^[22,23] SNPs in several genes, particularly genes responsible for lipid and glucose metabolism, insulin secretion, and insulin resistance, have been associated with GDM. These genetic variants are potential biomarkers; however, individually, they are likely to lack sensitivity.

miRNAs are specialized short non-coding RNAs approximately 22 nucleotides in length. Functionally, mature miRNAs guide the RNA-induced silencing complex to recognize target mRNAs at 3' untranslated regions, thus regulating gene expression.^[24-26] miRNAs are promising biomarkers of diseases owing to their high stability in body fluids and their enrichment in particular tissues. miRNAs have been associated with GDM and its health complications and may have diagnostic, prognostic, and predictive value.^[27-29] Three common methods for miRNA analysis are miRNA sequencing, microarray, and quantitative real-time PCR (qRT-PCR).^[30] miRNA sequencing utilizes next-generation sequencing for high-throughput analysis of miRNA. Though it has advantages of sensitivity, accuracy, and repeatability, it is also time consuming and costly.^[31] Microarray is also a technology for multiplex analysis of miRNAs. However, the relatively low sensitivity and specificity of microarray could be a challenge.^[32] qRT-PCR is currently the most widely used detection technology for miRNAs.^[33] Sensitivity, specificity, and dynamic range of qRT-PCR are considered as excellent, but it is useable only for miRNAs that have been previously identified and not suitable for discovery studies.^[34] Despite of its imperfections, qRT-PCR is the most common method for routine testing in the clinical laboratory because it is performed on standard equipment.^[35]

Proteomics is the large-scale analysis of peptides or proteins in cells, tissues, and body fluids and has attracted substantial attention over the past few decades owing to the roles of proteins in almost all biological processes.^[36] Four major approaches are involved in quantitative proteomics, including gel-based, stable isotope labeling, label free, and targeted proteomics. One-dimensional gel electrophoresis, two-dimensional polyacrylamide gel electrophoresis, and difference gel electrophoresis approaches have been utilized for protein separation in gel-based proteomics.^[37,38] In labeling proteomics, stable isotope labeling by amino acids in cell culture, isobaric tag for relative and absolute quantitation, and tandem mass tag are commonly used.^[39] Selected reaction monitoring and multiple reaction monitoring are MS-based techniques that act as reliable quantitation methods in targeted proteomics. A triple quadrupole mass spectrometer is employed to detect and analyze targeted proteins. Using this instrument, specific peptides representing each of the targeted proteins are selected based on their mass to charge (m/z) ratio, and they subsequently fragment into smaller ions for quantitation analysis.^[40,41] Label-free

proteomics is not restricted to labels and consequently reduces the overall experimental error. It also enables the comparison of protein expression among a great number of samples from various sources. MS has been invaluable for the revolution of proteomics, for example, stable isotope labeling coupled with MS-based techniques tremendously improved sensitivity and protein detection in comparison to classical approaches.^[42,43] Several proteomics studies have identified potential biomarkers for GDM and its complications, although GDM is believed to share a similar mechanism with T2D. These protein biomarkers, such as transthyretin, serum retinol binding protein 4, and the apolipoprotein (Apo) superfamily, are related to insulin resistance, glycolipid metabolism, inflammatory pathways, and oxidative stress.^[44,45]

In this review, we searched for studies of metabolites, SNPs, miRNAs, and proteins in human subjects with GDM published between January 2010 and September 2021. PubMed was systematically searched using search terms “gestational diabetes mellitus” or “postpartum diabetes” combined with “metabolite,” “single-nucleotide polymorphism,” “microRNAs” or “protein” and corresponding synonyms and associated terms for each word. Articles published in languages other than English were excluded. We describe altered biomolecule profiles, summarize the mechanisms of action of specific biomolecules, and discuss their use as biomarkers in the occurrence and development of GDM and postpartum diabetes. The main findings are summarized in [Figure 1].

Metabolomics in GDM

The identification of GDM-related metabolic biomarkers is important for early intervention and may improve our understanding of the underlying mechanisms. Blood (plasma and serum), urine, and hair are frequently utilized in metabolomic studies of GDM. In particular, blood samples are the most commonly used biofluids because blood is abundant and highly dynamic; however, sampling requires invasive procedures.^[46] Elevated levels of linoleic acid, alanine, leucine, lysophosphatidylcholine, tyrosine, phenylalanine, carnitine, and derivatives of cholic acid have

been consistently reported among patients with GDM.^[47-57] In contrast, decreases in serine,^[47,58,59] glutamine,^[58-60] and methionine^[59,60] have been observed.

Most studies have reported that levels of circulating BCAAs, which consist of valine, leucine, and isoleucine, were increased in patients with GDM.^[47,48,56] Increased levels of ketone bodies in GDM inhibit proteolysis and reduce the oxidation of BCAAs in skeletal muscle; thus, they are released at low rates from skeletal muscle and are mostly catabolized in the liver.^[61] The activities of BCAA catabolic enzymes in the liver and adipose tissue are repressed, contributing to the high blood concentration of BCAAs.^[62] Valine levels were also higher in infants of mothers with GDM than in infants of healthy mothers.^[63] Among children with GDM, daily intake of BCAAs was associated with elevated risks of overweight and insulin resistance; however, this association was not fully independent of daily energy intake in children.^[64] BCAAs are predictive of GDM and are associated with insulin resistance.^[65,66] It has been proposed that BCAA metabolism may contribute to insulin resistance because the accumulation of toxic BCAA metabolites could result in β -cell mitochondrial dysfunction and high susceptibility to insulin resistance.^[67] Another explanation for the association was that BCAAs activated the mammalian target of rapamycin pathway and phosphorylated insulin receptor substrate 1, thereby interfering with insulin signaling.^[68] BCAAs can also participate in glucose uptake by increasing the translocation of the glucose transporter 1 (GLUT1) and GLUT4 to the cell surface.^[69]

The area under the curve (AUC) is a typical parameter used to assess the predictive efficiency of potential biomarkers. In analyses of blood samples, a β -muricholic acid-based model had the best performance for the prediction of GDM, with the highest AUC (>0.95) and Youden index (>0.80) and a sensitivity of 92.1% and specificity of 96.3%.^[55] β -Muricholic acid is an intermediate in the metabolism of cholesterol to tauro- β -muricholic acid in the liver. Tauro- β -muricholic acid is then exported to the intestine and hydrolyzed to β -muricholic acid by bile salt hydrolase.^[70,71] As a potent farnesoid X receptor antagonist, tauro- β -muricholic acid inhibits intestinal farnesoid X receptor signaling, contributing to improved hepatic steatosis, insulin sensitivity, and glucose tolerance.^[72,73]

While blood provides a snapshot of the metabolic status of individuals at the time of sampling, urine represents a summation of process occurring in the hours prior to sampling.^[46] The acquisition of urine samples is non-invasive; however, the composition and volume depend on dietary habits and other factors. Women with GDM showed significant increases in urine levels of serotonin,^[19,74] tryptophan,^[19,54,75] glucuronide derivatives,^[75,76] and phenylalanine.^[54,77] Studies have also identified decreases in metabolites, including ethanolamine, lanthionine, 5-methoxytryptamin, threonine, methionine, methionine sulfoxide, acadesine, carnitine, argininate, and melatonin.^[19,54,60,76] The AUC values for these factors ranged from 0.718^[60] to 0.993,^[76] demonstrating good predictive performance. In one of the studies

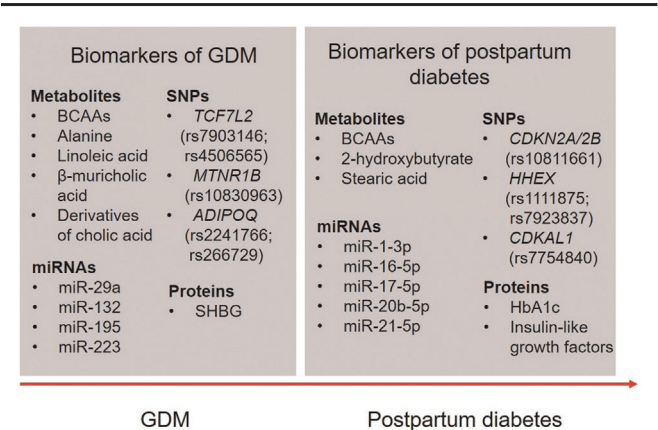


Figure 1: Biomarkers for GDM and postpartum diabetes. BCAAs: Branched-chain amino acids; GDM: Gestational diabetes mellitus; HbA1c: Hemoglobin A1c; miRNAs: microRNAs; SHBG: Sex hormone-binding globulin; SNPs: Single-nucleotide polymorphisms.

with the highest predictive performance, untargeted and targeted metabolomics approaches were used to explore plasma and urine sample metabolites, yielding an AUC for GDM prediction (combined with BMI) of 0.99.^[19] Another recent study of urinary metabolites in early pregnancy involving 46 women with GDM and 46 age-matched individuals without GDM applied a classification tree analysis based on saccharopine, dihydroorotate, nicotinate ribonucleoside, 7,8-dihydroneopterin, phenyl-glucuronide, lanthionine, and arginine and the AUC for the prediction of progression to GDM was 0.993.^[76]

Hair is easy to obtain in a non-invasive manner and highly stable for retaining long-term information; thus, it is a useful source of biomarkers.^[78] Analyses of hair samples are limited; however, increases in 2-aminobutyric acid and adipic acid have been detected.^[79,80] Further studies of hair samples are needed to confirm these findings and to identify more differentially expressed metabolites in women with GDM.

Approximately, 50%–60% of patients with GDM develop dysglycemia (T2D and prediabetes) after delivery;^[81,82] accordingly, a predictive test for this transition may improve patient management. Increases in BCAAs are predictors of the incident T2D risk after pregnancy complicated by GDM.^[83–85] Six years after GDM, levels of BCAAs and the valine metabolite 3-hydroxyisobutyrate were higher in women who developed T2DM, and there was a tendency for BCAA levels to increase in the impaired glucose tolerance group.^[85] Dudzik *et al*^[86] found that 2-hydroxybutyrate and stearic acid showed the best discriminative power (AUC of 0.90) for postpartum diabetes. Elevated levels of 2-hydroxybutyrate, which result from enhanced lipid oxidation, glutathione synthesis, and a redox imbalance,^[86] are associated with insulin resistance and reduced insulin secretion.^[87] These metabolite changes may be considered prognostic biomarkers for the prediction of postpartum diabetes among women with prior GDM.

Genomic Analysis of GDM

Since GDM shares pathophysiological similarities with T2D, some T2D-related genes are also important in the GDM process. The *TCF7L2* gene, which is highly associated with T2D predisposition, encodes a transcription factor that operates at the end of the Wnt signaling cascade not only in β cells but also in other organs, including the liver.^[88] rs7903146, rs4506565, rs7901695, rs12255372, and rs12243326 in the *TCF7L2* gene are associated with GDM. As the most frequently reported polymorphism in *TCF7L2*, rs7903146 was associated with an increased risk of GDM.^[89–95] In addition, patients with GDM harboring the T risk allele in rs7903146 were more likely to have early postprandial glycemic control failure and require insulin therapy during pregnancy.^[96] rs7903146 is located in islet-selective open chromatin, indicating that the chromatin state at rs7903146 is more open in chromosomes carrying the T allele, allowing the binding of regulatory proteins to this locus in human islet cells.^[97] Previous studies have shown that it was related to impaired insulin secretion, decreased incretin effects, and

hepatic insulin resistance.^[98,99] Pilgaard *et al*^[99] found that risk allele carriers showed an increased proinsulin/insulin ratio, reduced insulinotropic effects of incretin hormones, and impaired β -cell responsiveness to glucose. Cropano *et al*^[100] also revealed that the T allele of rs7903146 contributed to the development of hyperglycemia by altering the proinsulin secretory efficiency and reducing the ability of insulin to suppress hepatic endogenous glucose production. The T allele of rs4506565 was also a risk allele for GDM.^[92,93,101] Pagan *et al*^[101] found that TCF7L2 rs4506565 was associated with a two-fold increase in the risk of developing GDM. Pregnant individuals carrying this risk allele had elevated levels of resistin, a member of the adipokine family, in the plasma and cord blood. Additionally, the allele was associated with increased interleukin (IL)-6 levels, suggesting that it had an effect on GDM via inflammation.

MTNR1B encodes the melatonin MT2 receptor, also known as melatonin receptor type 1B. As a member of the G-protein-coupled receptor superfamily, it affects glucose intolerance and insulin resistance.^[102] Four SNPs in the *MTNR1B* gene were frequently reported, including rs10830963. Most studies of rs10830963 have shown that the G allele was the risk allele and was correlated with increased fasting glucose, increased hemoglobin A1c (HbA1c), and an impaired early insulin response to glucose.^[103–105] Glucokinase (GK) is a tissue-specific enzyme mainly present in the liver and pancreatic islets,^[106] where glucose is phosphorylated by GK into glucose-6-phosphate for further glycometabolism.^[107] Individuals carrying the G allele showed higher expression of *MTNR1B* via increased FOXA2-bound enhancer activity and neuronal differentiation 1 (*NEUROD1*) binding in islet cells,^[108] and melatonin lowered intracellular cyclic adenosine monophosphate (cAMP) levels, leading to the downregulation of GK expression and impaired insulin secretion.^[105,109] In an analysis of individuals with a high GDM risk (i.e., with a history of GDM and/or BMI ≥ 30 kg/m²), only non-carriers of the risk allele (i.e., G) benefited from lifestyle interventions, suggesting that the *MTNR1B* rs10830963 variant could modify the efficacy of lifestyle interventions.^[110] Moreover, for patients with GDM with a pre-pregnancy BMI ≥ 29 kg/m² carrying the rs10830963 G risk allele, a study reported that despite the medical nutrition therapy and lifestyle intervention, endogenous insulin secretion was insufficient to meet the increased insulin demand, and antenatal insulin therapy initiation may be necessary.^[111]

The *ADIPOQ* gene spans 17 kb on chromosomal region 3q27 and encodes human adiponectin, which is secreted mainly by adipose tissue. This adipocyte-derived plasma protein can reverse insulin resistance and increase insulin efficacy in glucose metabolism.^[112] It is related to obesity, T2D, and metabolic syndrome.^[113] rs1501299, rs2241766, and rs266729 are the most frequent SNPs. Although studies of rs1501299 have not detected an association with GDM,^[114,115] most studies of rs2241766 have shown that the G allele was associated with an increased risk of GDM.^[116–119] Several studies have indicated that subjects with the rs2241766 G allele showed a decrease in

adiponectin levels and an increase in the insulin resistance index.^[120,121] Considering that rs2241766 is a silent polymorphism with no impact on the sequence of amino acids, it is possible that this SNP inactivates the gene by influencing transcript activity, such as the splicing accuracy or efficiency.^[122] The association between rs266729 and GDM remains inconclusive.^[114,115,123,124] Conflicting results can be explained by differences in environmental factors and lifestyle among populations. In addition, inconsistent detection methods and sample sizes might have contributed to the differences.

Relatively few studies have evaluated the genetic risk of postpartum diabetes among women with GDM. Notably, rs10811661 of *CDKN2A/2B* and rs1111875 and rs7923837 of *HHEX* increased the risk of early conversion (2 months), whereas rs7754840 of *CDKAL1* increased the risk of late conversion (more than a year).^[125] Some SNPs associated with postpartum glycemic traits have been identified in women with a history of GDM. *MTNR1B* rs10830963 was genotyped in 1025 women with previous GDM, revealing its relationship with postpartum fasting glucose levels. In a stratified analysis, the *MTNR1B* genotype was related to postpartum changes in the 2-hour oral glucose tolerance test (OGTT) across categories of inadequate, adequate, and excessive gestational weight gain.^[126] Similarly, rs10830963 and rs1387153 of *MTNR1B* were associated with elevated fasting glucose levels in another study, with highly significant *P* values.^[90] In a study of 1208 women with prior GDM, *MC4R* rs6567160 was not significantly associated with postpartum fasting glucose but was positively associated with 2-hour OGTT glucose concentrations and increased HbA1c.^[127]

The genetic risk score (GRS) is computed as the sum of unweighted or weighted risk variants. This parameter combines genetic information for multiple variants and thereby has high predictive power. Given the relatively small effect sizes of individual risk loci, the GRS includes SNPs at independent loci to predict the genetic risk of diseases. A GRS based on 48 genetic variants showed good prediction performance for T2D in women with GDM; women who developed diabetes after GDM pregnancy had a higher unweighted and weighted GRS (wGRS) than those who did not meet the diabetes diagnostic criteria. The risk of diabetes increased as the quartiles of wGRS increased. The hazard for diabetes incidence was 5.52 times higher in the highest wGRS quartile than in the lowest wGRS quartile. In a complex clinical model for the prediction of future diabetes, the *c*-statistic increased from 0.741 without the wGRS to 0.775 with the wGRS and the net reclassification improvement index was 0.430.^[128] Another GRS model based on 36 SNPs was also predictive of pre-diabetes and T2D in women with previous GDM; when the explained-variance GRS was added to a model including age and BMI, the AUC increased from 0.6269 to 0.6672, indicating an improved predictive value.^[129]

miRNAs in GDM

There is substantial interest in the roles of miRNAs in the pathogenesis of GDM. In the last few years, a number of

miRNAs that are upregulated in women with GDM have been identified, including miR-16-5p,^[130-132] miR-19a,^[132,133] miR-19b,^[132,133] miR-101,^[134,135] miR-137,^[136,137] miR-195,^[134,138-140] miR-223,^[141,142] miR-330-3p,^[28,143,144] miR-342-3p,^[134,145] and miR-657.^[146,147] Like many other miRNAs, miR-223 has been implicated in various physiological and pathological conditions, including inflammatory disorders,^[148,149] infection,^[150,151] and cancer.^[152,153] It was downregulated in T2D^[154] but was upregulated in the insulin-resistant heart of patients with T2D.^[155] Independent of phosphoinositide 3-kinase signaling or AMP kinase activity, miR-223 increased glucose uptake via GLUT4 protein expression in cardiomyocytes.^[155] It was speculated that the overexpression of miR-223 in the insulin-resistant heart is a compensatory mechanism for the systemic reduction of miR-223.^[156] However, miR-223 was upregulated in GDM, and a prediction model based on miR-223 alone had a better accuracy than that of a model including the differentially expressed miRNAs miR-223 and miR-23a, with an AUC value of 0.94 and accuracy of 0.90.^[141] Another model based on miR-223 showed a similar predictive value (AUC = 0.92).^[142] Serum expression of miR-29a was significantly downregulated in pregnant women with GDM compared with healthy women, and its expression decreased ahead of the elevation of serum glucose.^[157] However, the AUC for miR-29a varied greatly, from 0.658^[157] to 0.829.^[158] The knockdown of miR-29a increases the expression of *Insig1* gene and subsequently enhances the level of phosphoenolpyruvate carboxy kinase 2. As a key enzyme in gluconeogenesis and glycolysis, phosphoenolpyruvate carboxy kinase 2 expression may lead to increased glucose level.^[157] miR-29a is not only a potential regulator of serum glucose but also a negative regulator of cannabinoid type 1 receptor. Overexpressing miR-29a inhibits the expression of proinflammatory and profibrogenic mediators, preventing diabetic glomeruli damage caused by fibrosis.^[159]

Other miRNA-related mechanisms contributing to the pathogenesis of GDM have been discovered. For example, miR-657 could promote macrophage proliferation, migration, and polarization toward the M1 phenotype by downregulating family with sequence similarity 46 member C, thus effecting macrophage-mediated immunity and inflammation in GDM.^[147] The downregulation of miR-770-5p could enhance pancreatic β -cell proliferation, promote insulin secretion, and suppress cell apoptosis via the TP53 regulated inhibitor of apoptosis 1 (TRIP1)/apoptotic peptidase activating factor 1 (APAF1) pathway; hence, the miRNA plays a protective role in GDM.^[160] miR-96 also contributes to β -cell proliferation and function by targeting and downregulating p21-activated kinase 1.^[161] The downregulation of miR-29b may be partially related to the development of GDM by increasing the expression of hypoxia-inducible factor 3A, promoting trophoblast cell activity.^[162] Serum aberrant expression of miR-132 in GDM is observed prior to glucose abnormality,^[157] and miR-132 may exert a protective role against GDM through abrogating the inhibiting effects of high glucose on trophoblast cell proliferation.^[163] The dysfunction of vascular endothelial cells could account for the high risk of cardiovascular diseases in patients with GDM

and their offspring. The overexpression of miR-137 induces human umbilical vein endothelial cell dysfunction under high glucose conditions by promoting the secretion of CC chemokine ligand-2 (CCL2)/monocyte chemoattractant protein-1 (MCP-1), upregulating the levels of IL-6, intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, and downregulating IL-8 and vascular endothelial growth factor.^[136] By the inhibition of the target gene peroxisome proliferator-activated receptor- α , miR-518d may disrupt the homeostatic balance between cellular fatty acid and glucose metabolism and increase resistance to insulin.^[164] These findings clearly establish the importance of miRNAs in the development of GDM, suggesting that further investigations of miRNAs may shed light on the pathophysiology of the disease.

In addition to associations between miRNAs and the risk of GDM, some investigators have evaluated trimester-specific miRNA changes in cases and controls. Herrera-Van Oostdam *et al*^[130] found that in the second trimester, miR-517-3p and miR-518-5p levels were higher in cases than in controls, while the opposite pattern was observed in the third trimester. Lamadrid-Romero *et al*^[137] also observed that the level of miR-125b-5p increased in the first trimester and decreased during the second trimester. Additionally, levels of miR-200b-3p and miR-183-5p, which were upregulated in the first and second trimester, respectively, were downregulated in the third trimester. These findings may be helpful in tracking disease progression and clarifying the pathophysiology.

Other studies of the risk of developing diabetes after delivery among women with a history of GDM based on miRNAs are lacking. However, Hromadnikova *et al*^[165] assessed the risk of later development of diabetes mellitus and cardiovascular and cerebrovascular diseases in 111 women with GDM and 89 age-matched women after normal pregnancies. They selected a series of miRNAs involved in the pathogenesis of diabetes mellitus and cardiovascular and cerebrovascular diseases. The expression levels of these miRNAs were higher in women with a history of GDM than in the control group. Among these miRNAs, miR-1-3p, miR-16-5p, miR-17-5p, miR-20b-5p, miR-21-5p, miR-23a-3p, miR-26a-5p, miR-29a-3p, miR-103a-3p, miR-133a-3p, miR-146a-5p, miR-181a-5p, miR-195-5p, miR-199a-5p, miR-221-3p, and miR-499a-5p showed good sensitivity at a 10.0% false positive rate and high accuracy for the identification of women previously affected with GDM at a higher risk of postpartum diabetes mellitus and cardiovascular and cerebrovascular diseases. These miRNAs were highly discriminative, with an AUC of 0.900, sensitivity of 77.48%, and specificity of 93.26%.

Proteomics in GDM

Protein biomarkers in women with GDM included immune molecules, hormones, enzymes, polypeptides, and glycoproteins. Sex hormone-binding globulin (SHBG) is a glycoprotein synthesized mainly in the liver that binds to and regulates sex steroids with high affinity and specificity.^[166] Low concentrations of SHBG are a biomarker for early GDM.^[167,168] Even pre-pregnancy SHBG levels are a predictor of subsequent GDM; women

with SHBG levels below the median appeared to have a 2.6-fold increased risk for GDM. A greater effect of low SHBG levels was observed in women who were overweight or obese (BMI ≥ 25.0 kg/m²), with a 5.3-fold increased risk of GDM compared with that of normal weight women with high SHBG concentrations.^[169] Despite controversies regarding the inhibitory effects of insulin on SHBG production,^[170-172] there is evidence for an association between SHBG levels and the development of insulin resistance.^[173,174] Some studies have attempted to explain the inverse association. For instance, Wang *et al*^[175] speculated that SHBG may exert its biological effects by inhibiting the extra-cellular signal-regulated kinase (ERK) pathway, thus influencing insulin secretion and participating in the onset of insulin resistance and GDM. Disruption of the ERK isoform ERK1 in mice resulted in resistance to high-fat diet-induced obesity and improved insulin sensitivity.^[176] Activation of the ERK pathway by IL-1 β could decrease insulin-induced glucose transport mainly by inhibiting insulin receptor substrate 1 expression at the transcriptional level.^[177] In addition, Chi *et al*^[178] found that SHBG in the placenta may regulate the expression of GLUT1 via the activation of the cAMP/protein kinase A (PKA)/cAMP responsive element binding protein 1 (CREB1) pathway, thereby affecting glucose metabolism and improving insulin resistance.

In addition to differences in protein expression levels between patients with GDM and healthy women, longitudinal changes in differentially expressed proteins have been investigated. In a prospective cohort study, serum proteins were screened in the early stage (12–16 weeks) and middle stage (24–28 weeks) of pregnancy in 60 participants (30 GDM cases and 30 healthy controls). In total, 31 and 27 proteins were differentially expressed between GDM cases and controls in the early and middle stages, respectively. When compared with the early stage, 38 and 28 proteins were altered in the middle stage in healthy controls and patients with GDM, respectively, and these proteins may be associated with the progression of normal pregnancy and GDM.^[179] Among these proteins, beta-ala-his dipeptidase was highly discriminative (AUC = 0.98),^[180] whereas Apo E was less discriminative (AUC = 0.965). The combination of Apo E, coagulation factor IX, fibrinogen alpha chain, and insulin-like growth factor-binding protein 5 increased the AUC to 0.985, with 80% sensitivity and 95% specificity.^[181]

An increasing number of studies have focused on the clinical value of HbA1c levels in predicting the development of postpartum diabetes among patients with GDM. A logistic regression analysis indicated that HbA1c $\geq 5.4\%$ was associated with a 5.5-fold increased risk of postpartum diabetes.^[182] In another study, at the optimal HbA1c cutoff value of 5.55%, the AUC was 0.846, with 78.6% sensitivity and 72.5% specificity.^[183] Coetzee *et al*^[184] investigated the value of HbA1c levels at the time of GDM diagnosis (t1) and in the 4 weeks preceding delivery (t2) for the prediction of postpartum diabetes. The receiver operating characteristic curve analysis indicated that HbA1c at GDM diagnosis performed well (AUC = 0.90). At a cutoff of 6.2%, the sensitivity and specificity were 95% and 62%, respectively. The optimal

cutoff value for HbA1c in the 4 weeks preceding delivery was 6.2%, with an AUC value of 0.81, and patients with GDM and HbA1c $\geq 6.2\%$ had a four-fold (at t1) or five-fold (at t2) increased risk of diabetes, respectively. Thus, HbA1c could be used as a tool to predict postpartum diabetes in women with GDM.

Insulin-like growth factors and their binding proteins are correlated with the development of T2D. Lappas *et al.*^[185] further showed that postpartum insulin-like growth factor-binding protein-2 levels were significantly and negatively associated with the development of T2D among women with previous GDM, even after adjusting for age and BMI. In contrast, insulin-like growth factor I levels were positively associated with postpartum T2D. When the above two factors were added to a base model (including age, BMI, fasting glucose, and postnatal fasting glucose), the predictive model identified individuals with postpartum diabetes with an AUC of 0.892. Similarly, in 2019, Lappas *et al.*^[185] explored the relationship between postpartum diabetes and Apo species based on their roles in T2D. Apo CIII levels as well as the Apo CIII/Apo AI, Apo CIII/Apo AII, Apo CIII/Apo CII, Apo CIII/Apo E, and Apo E/Apo CIII ratios were positively associated with the development of diabetes; when these parameters were added to the base model, the accuracy (83.2% to 86.3%), sensitivity (30% to 40%), and AUC values (0.782–0.824) were improved.^[186] Thus, these variables were identified as risk factors for the prediction of T2D in women with prior GDM.

Challenges and Future Directions

Several biomolecules have recently emerged as biomarkers for GDM and postpartum diabetes and can offer a basis for early diagnosis and targeted treatment. Biomarkers, including metabolites, SNPs, miRNAs, and proteins, are involved in glucolipid metabolism, insulin resistance, and inflammation. Thus, a complex network linking these factors should exist to integrate the biological information. However, the results of some GDM studies are highly inconsistent and difficult to replicate. Sample size and demographic factors are major bottlenecks in identifying credible diagnostic and prognostic biomarkers of GDM. In addition, most studies are case-control studies; these retrospective analyses do not provide insight into prevalence or incidence. Therefore, prospective studies are needed to explore the associations between biomarkers and GDM in a more powerful way. More importantly, while most biological samples are collected in the second trimester, at the time of GDM diagnosis, samples collected prior to the existence of GDM or at its onset are more critical for early prediction and diagnosis. The widely accepted approach for GDM diagnosis is 75 g OGTT performed during 24–28 gestational weeks, which seems a little late for intervention and prevention. In this sense, biomarkers released in the first trimester or ahead of glucose abnormality may help identify women at high risk of GDM and early GDM. However, relatively few studies target this area and no single molecule currently performs sufficiently well to be an established screening tool for early prediction and diagnosis of GDM. Though women with GDM are recommended to screen for T2D after

pregnancy,^[187] the compliance among this group is relatively low probably due to lack of awareness of the need for screening and the time-consuming nature of the tests.^[188] Since some pathophysiological changes occur long before the elevation of blood glucose, it is possible to build up a more accurate and acceptable test based on biomolecules for the prediction of T2D following GDM pregnancy. However, existing studies are mostly limited to certain racial groups and short-term follow-up.

Hence, biological samples obtained at different stages of pregnancy would be of great use in exploring the etiology and prognosis of GDM. Biomolecules, especially those released in the early stage, need to be tested on larger and more diverse populations to assess their predictive value. Further research with a much longer follow-up period may help explore their potential utility as screening tools, considering sensitivity, specificity, cost, and patient acceptability. Since the mechanism underlying the pathological progression of GDM is not entirely clear, the integration of data from various omics-based approaches is crucial, although numerous challenges remain.

Conflicts of interest

None.

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