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Screening for Gestational Diabetes Mellitus: The Potential of MicroRNAs

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Abstract

Gestational diabetes mellitus (GDM) is associated with short- and long-term complications in both mothers and their offspring. Screening and early diagnosis of GDM is advocated as a strategy to prevent adverse pregnancy outcomes. However, there is currently no test that is amenable to routine screening, particularly in low- and middle-income countries. MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression post-transcriptionally. In recent years, miRNAs have been the focus of increasing research due to their important role in regulating biological pathways and their aberrant expression during disease. The discovery of circulating miRNAs in maternal blood, and their altered expression during pregnancy-associated complications have increased interest into their potential as diagnostic biomarkers for GDM. In this review, we summarise studies that have investigated miRNAs in maternal blood thus providing an update of the current status of miRNAs as biomarkers for GDM. We also discuss the challenges of miRNA profiling, and highlight perspectives and recommendations for research.

Keywords: gestational diabetes mellitus, biomarkers, epigenetics, microRNAs, pregnancy

1. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that is first diagnosed during pregnancy with glucose homeostasis usually restored shortly after birth. The rate of GDM has constantly increased over the last 20 years [1], and in 2017 the International Diabetes Federation estimated that about 14% of women with live births had GDM [2]. Without appropriate glucose management, GDM is associated with short- and long-term complications in both mothers and their offspring [3–6]. Treatment of GDM is effective in preventing these adverse outcomes [7–11], thus, universal screening and early detection of GDM is widely advocated as a strategy to promote timely treatment and improve pregnancy outcomes [3]. The oral glucose tolerance test (OGTT) conducted between the 24th and 28th week of pregnancy, is currently the gold standard for GDM diagnosis [12]. However, the test is time-consuming, expensive and unfeasible in most countries. The identification of simple and cost-effective biomarkers that do not require fasting and multiple blood draws would be more acceptable to pregnant women, and thereby facilitate

screening for GDM. MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate various metabolic pathways. They are implicated in the pathophysiology of various diseases and have attracted considerable interest as biomarkers of metabolic disease. Recently, several studies have explored their potential as biomarkers of GDM. The purpose of this review is to provide an update of the status of miRNAs as biomarkers for GDM. All studies that have profiled miRNAs in maternal blood during GDM to date are summarised. We also discuss the challenges of miRNA research, and highlight perspectives and recommendations for future research.

2. Overview of gestational diabetes

Hyperglycaemia during pregnancy creates an adverse intrauterine environment that predisposes both mother and offspring to perinatal complications and future metabolic disease [3–6]. Maternal perinatal complications include caesarean section, preeclampsia and birth injuries. Women with pregnancies complicated by GDM also have an increased risk of developing disease in later life. In 2009, Bellamy et al. conducted a comprehensive review of the literature and found that women who have had GDM are at least seven-fold more likely to develop Type 2 diabetes (T2D) compared to women with normoglycaemic pregnancies [4]. Other studies showed that GDM is associated with the development of metabolic disease [13], cardiovascular disease [14] and breast cancer [15].

Foetal and neonatal complications associated with GDM include macrosomia, congenital malformations, perinatal death, hypertrophic cardiomyopathy, intrauterine growth restriction, preterm birth, respiratory distress syndrome, hypoglycaemia, hypocalcaemia, polycythaemia and hyperbilirubinemia [5]. In recent years, increasing evidence support the critical role of the intrauterine environment in programming the foetus and influencing long-term offspring health [16]. In the 1980s, David Barker and his colleagues proposed *Barker's hypothesis or the developmental origins of adult disease*, which suggests that metabolic diseases have their origins in early development [17]. Subsequently, several other studies have reported that diabetes during pregnancy is associated with the development of obesity and diabetes in children [5].

The prevalence of GDM is rapidly increasing, spurred by the global obesity pandemic. Pregnant women who are overweight, obese or severely obese have a 2.14-, 3.56- and 8.56-fold risk of developing GDM compared to normal weight women [18]. The short- and long-term consequences of GDM are likely to have a major negative impact, particularly on low- and middle-income countries that already have limited financial and human resources, and are least able to respond to the challenge. Screening and treatment of GDM leads to improved pregnancy outcomes [7–11], thus universal screening for GDM is widely advocated as a strategy to prevent pregnancy complications. However, the OGTT, which is considered the gold standard for GDM diagnosis is not amenable to routine screening [3]. Currently, traditional risk-factor screening based on obesity, age older than ≥ 35 years, non-white ethnicity, and having a family history of diabetes [3] is mostly employed. Unfortunately, these risk factors have poor predictive value [19, 20]. A number of other laboratory tests such as glycated haemoglobin (HbA1c), insulin, adiponectin, glycosylated fibronectin and C-reactive protein have been explored, however, they too have several challenges and are not yet clinically applicable [3].

3. Characteristics of ideal biomarkers

Biomarkers are defined as “cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells or fluids” [21].

Screening, diagnostic and prognostic biomarkers offer several advantages and thus efforts to identify biomarkers of disease have intensified. They are clinically useful and can be used to detect or monitor disease progression, thus facilitating earlier diagnosis and disease management. Furthermore, biomarkers are able to monitor pharmacological responses and predict clinical outcome. As recently reviewed by Etheridge et al. [22], characteristics of the ideal biomarker include sensitivity, specificity, cost-effectiveness, reproducibility, robustness, accessibility, stability and ability to differentiate between pathologies. Recent advancements in molecular biology have led to the development of molecular biomarkers that are sensitive and specific, and are easily measured in biological fluids such as whole blood, plasma and serum [22].

4. MicroRNAs

MiRNAs are epigenetic mechanisms that reflect gene-environment interactions and are increasingly being implicated in the pathophysiology of metabolic diseases [23]. Since their discovery in *Caenorhabditis elegans* in 1993 [24], miRNAs have emerged as one of the most powerful epigenetic mechanisms regulating diverse biological processes including development, proliferation, differentiation and apoptosis [25]. They are short, single-stranded, highly conserved, non-coding RNA molecules of approximately 22 nucleotides in length that regulate gene expression through post-transcriptional mechanisms. MiRNAs bind to the 3' untranslated region (UTR) of messenger RNA (mRNA) inducing degradation or translational repression of the mRNA transcript [26]. Using an elegant set of experiments, Guo et al. showed that destabilisation of target mRNAs rather than translational repression is the main mechanism whereby miRNAs reduce protein expression [26]. A single miRNA is able to regulate up to 200 target genes, implying that about 30% of the genome is regulated by miRNAs [27, 28] and confirming the important role of miRNAs as mediators of biological function. More than 2000 miRNAs are present in the human genome, and function in various biological processes [27–29].

Mature miRNAs are produced through a stepwise process. Briefly, primary miRNA transcripts (pri-miRNAs) are transcribed in the nucleus by RNA polymerase II (and possibly by RNA polymerase III), which are then cleaved by Drosha RNase III endonuclease to produce stem-loop precursor miRNAs (pre-miRNAs) that are approximately 70 nucleotides long. Ran-GTP and the export receptor, Exportin-5 transports pre-miRNAs to the cytoplasm, where Dicer, also a RNase III endonuclease, cleaves them to produce mature miRNAs. Mature miRNAs complex with the RNA-induced silencing complex (RISC) and bind to the 3' UTR of mRNA to induce predominantly mRNA degradation [25, 26].

MiRNAs regulate a wide range of biological processes including cell proliferation and differentiation, apoptosis and metabolism, thus it is not surprising that altered miRNA expression have been shown to associated with various conditions including cancer, obesity, T2D and cardiovascular disease [30]. MiRNAs play a critical role in the pathophysiology of metabolic disease, and their aberrant expression is observed in tissues associated with disease. For example, various *in vitro*, *in vivo* animal models and studies in diabetic patients have demonstrated the altered expression of miRNAs that regulate insulin secretion, adipocyte differentiation, lipid metabolism, inflammation and glucose homeostasis in dysfunctional pancreatic beta cells and insulin-resistant target tissues, such as adipose, liver and muscle during T2D [23]. Increasingly evidence show that correcting aberrant miRNA expression can prevent or treat T2D, making them attractive therapeutic targets [31].

The identification of circulating miRNAs in biological fluids such as whole blood, serum, plasma and urine has sparked research efforts to investigate their

feasibility as diagnostic or prognostic biomarkers of disease [32–34]. Circulating miRNAs are speculated to reflect tissue expression, to play a central role in cell-to-cell communication and to be associated with disease progression [33–35]. Other attributes that make miRNAs attractive biomarkers is their stability and robust expression [22], even in degraded RNA samples [36]. Technological advances and the development of various platforms for miRNA profiling have bolstered the popularity of miRNAs [37], and have enabled relative easy and cost-effective methods of quantification using sensitive techniques such as quantitative real time PCR (qRT-PCR) [22, 32, 38]. Circulating miRNAs are thought to be released from cells as exosomes, microvesicles, apoptotic bodies, or are non-vesicle bound and encapsulated in protein or lipid complexes [32]. A number of studies [39–41], have demonstrated that circulating miRNAs are associated with glucose homeostasis and are dysregulated during T2D progression. Recently, we showed that the expression of miR-27b is increased in peripheral blood cells and serum of South African women with impaired glucose tolerance compared to normoglycaemia [42] and identified novel miRNAs associated with dysglycaemia in these women [43]. Putative gene targets of these novel miRNAs were enriched in biological processes involved in key aspects of glucose regulation, and receiver operating characteristic (ROC) curve analysis demonstrated that the diagnostic utility of these miRNAs were similar to fasting insulin [43]. Intriguingly, Parrizas et al. showed that an exercise intervention was able to reverse the aberrant expression of miR-192 and miR-193b induced by impaired glucose tolerance [44], while Luo et al. [45] showed that platelet-derived miR-126 was altered during T2D progression, and that glucose lowering treatment was able to normalise its expression. These studies provide support for the use of miRNAs as diagnostic and prognostic biomarkers to monitor treatment response.

5. MicroRNAs, pregnancy and gestational diabetes

MiRNAs play an important role as metabolic and developmental regulators during pregnancy. They respond to changing physiological conditions during pregnancy, while their dysregulation contributes to pregnancy-related disorders [46]. Thus far over 600 placental miRNAs have been identified [47]. Altered placental miRNA expression has been demonstrated in several pregnancy related disorders. In 2007, Pineles et al. were the first to demonstrate altered miRNA expression during preeclampsia. They reported that the expression of two miRNAs, miR-210 and miR-182, were increased during preeclampsia [48]. In 2009, using microarrays, Hu et al. and Zhu et al. identified seven and 34 miRNAs, respectively, that are dysregulated in preeclamptic compared to normal pregnancies [49, 50]. Subsequently, other studies have reported differential miRNA expression during preeclampsia and importantly provide experimental evidence to support the involvement of these miRNAs in disease pathophysiology [51, 52]. Altered miRNA expression has also been observed in other pregnancy complications such as macrosomia [53], preterm delivery and small for gestational age [54].

Placental-derived miRNAs in maternal blood have potential as biomarkers for pregnancy monitoring [54, 55]. It is suggested that miRNAs from placental tissue are exported into the maternal circulation via exosomes, and that these miRNAs reflect the physiological status of pregnancy and may thus have diagnostic potential [56]. Many of studies have reported that maternal circulating miRNAs are associated with placental weight [57], placental dysfunction [58, 59] and pregnancy complications [54, 60–63]. MiR-517c was increased in pregnancies complicated with placental abruption [59], miR-515, miR-516a, miR-516b, miR-518b, miR-519d, miR-520a, miR-520h, miR-525, miR-526b and miR-1323 were increased during

preeclampsia [63], miR-516, miR-517, miR-520a, miR-525 and miR-526a were upregulated during preeclampsia, gestational hypertension and foetal growth restriction [60], miR-517a was increased and miR-518b was decreased during placenta previa [58], and miR-346 and miR-582 were increased during preeclampsia, preterm delivery and small for gestational age patients compared to normal controls [54]. Importantly, Hromadnikova et al. showed that upregulation of plasma miR-517, miR-518b and miR-520h during the first trimester was associated with the development of preeclampsia, and provided evidence to suggest that miR-517 could predict preeclampsia [61]. Furthermore, a number of studies have reported that circulating miRNAs are associated with macrosomia [62, 64, 65]. Jiang and colleagues demonstrated that maternal expression of miR-21, and to a lesser extent miR-20a in serum samples from pregnant women in the third trimester was associated with macrosomia [62], Hu et al. reported that macrosomia was associated with decreased serum expression of miR-376a [64], while Ge et al. reported that the expression of miR-18a, miR-141, miR-143, miR-200c and miR-221 were decreased, and miR-16, miR-30a and miR-523 were increased in the plasma of pregnant women with foetal macrosomia compared to normal controls [65], further supporting the use of miRNAs as predictive biomarkers for pregnancy complications.

Growing evidence implicate miRNAs in the pathogenesis of GDM [47] and suggest that maternal miRNA expression may be used as biomarkers to predict GDM. Indeed, many GDM associated miRNAs are also expressed in placentas of women with Type 1 diabetes and T2D, confirming that miRNAs expressed during GDM play an important role in metabolic regulation and reflects some of the shared aetiology between these different types of diabetes [66]. Interestingly, a subset of miRNAs were distinct for each type of diabetes, illustrating their potential to differentiate between GDM and other manifestations of diabetes. Several other studies have reported that placental miRNA expression is altered in women with GDM. Zhao et al. reported that miR-518d is upregulated in placentas of women with GDM compared to controls, and further showed that increased expression of miR-518d correlated with decreased protein expression of peroxisome proliferator-activated receptor- α (PPAR α) [67], a major regulatory transcription factor in lipid homeostasis and energy metabolism [68, 69]. Li et al. identified nine miRNAs that are dysregulated in placentas of women with GDM, the expression of miR-508 was increased and miR-9, miR-27a, miR-30d, miR-33a, miR-92a, miR-137, miR-362 and miR-502 were decreased. Importantly, the decreased expression of these miRNAs correlated with increased protein expression of their gene targets, epidermal growth factor receptor (EGFR), phosphoinositide 3-Kinase (PI3K) and protein kinase B (Akt), key proteins in placental development and foetal growth [70]. Other studies showed that miR-98 [71] and miR-503 [72] are upregulated and miR-143 is downregulated [73] in placentas of women with GDM compared to women with normoglycaemic pregnancies. Intriguingly, the expression of miR-143 differentiated between GDM managed by diet or medication [71], further supporting the clinical value of miRNAs.

Xu et al. showed that the increased expression of miR-503 in the placentas of women with GDM compared to normoglycaemic pregnancies, are reflected in plasma [72]. The studies that have quantified circulating miRNA expression during GDM are summarised in **Table 1**. In 2011, Zhao et al. were the first to profile the expression of serum miRNAs during GDM [74]. Using Taqman low density arrays, followed by confirmation with individual qRT-PCR, they found that serum expression of miR-29a, miR-132 and miR-222 were decreased during GDM. Importantly, these results were validated in an internal and external cohort. Notably, serum for miRNA profiling in the discovery cohort was collected at 16–19 weeks of pregnancy, while GDM was diagnosed at 24–28 weeks of pregnancy, thus illustrating

GDM/ controls	Bio logical source	Detection method	Up- regulated	Down- regulated	No change	Normali sation	Ref
24/24 [*] (16– 19 weeks gestation)	Serum	Taqman low density array qRT-PCR	–	miR-29a miR-132 miR-222	–	Cel- miR-39	[74]
28/53 (13– 31 weeks gestation)	Serum	qRT-PCR	–	miR-20a miR-222	miR-16 miR-17 miR-19a miR-19b miR-29a miR-132	Cel- miR-39	[75]
13/9 (23– 31 weeks gestation)	Plasma	qRT-PCR	let-7e let-7 g miR-100 miR-101 miR-146a miR-8a miR-195 miR-222 miR-23b miR-30b miR-30c miR-30d miR-342 miR-423 miR-92a	–	–	Cel- miR-39	[76]
36/80 (7–23 weeks gestation)	Plasma	qRT-PCR	miR-155 miR-21		miR- 146b miR-517 miR-222 miR-210 miR-518a miR-29a miR-223 miR-126	Cel- miR-39 and miR-423	[77]
10/10 (16– 19 weeks gestation)	Plasma	Sequencing qRT-PCR	miR-16 miR-17 miR-19a miR-19b miR-20a	–	–	miR-221	[78]
85 GDM and 72 controls (16–20, 20–24 and 24–28 weeks gestation)	Plasma	qRT-PCR	miR-16 miR-17 miR-20a	–	miR-19a miR-19b	RNU6	[79]
21/10 (24– 33 weeks gestation)	Plasma	qRT-PCR	miR-330	–	miR- 548c	miR-374 miR-320	[92]
30/30 (24– 32 weeks gestation)	Whole blood	Sequencing qRT-PCR	miR-340	–	–	RNU6B	[93]
20/20 NK	Whole blood	qRT-PCR	–	miR-494	–	RNU6	[94]

GDM/ controls	Bio logical source	Detection method	Up- regulated	Down- regulated	No change	Normali sation	Ref
67/74 (16–20, 20–24 and 24–28)	Serum	qRT-PCR	miR-183 miR-200b miR-125b miR-1290	–	–	Cel- miR-39	[95]
11/12 (third trimester)	Plasma	qRT-PCR	miR-137	–	–	RNU6	[81]
25/25 NK	Plasma	qRT-PCR	miR-503	–	–	NK	[72]

*Validated in internal (36 GDM/36 controls) and two external cohorts (16 GDM/16 controls each).
GDM: gestational diabetes mellitus; qRT-PCR: quantitative real time polymerase chain reaction; NK: not known.

Table 1.
Studies investigating microRNA expression in maternal blood during gestational diabetes mellitus.

the potential of these miRNAs as screening tools for GDM. Recently, Pheiffer et al. reported that the expression of miR-29a, miR-132 and miR-222 were similarly decreased in the serum of South African women with GDM, however, only the latter was statistically significant [75]. Conflictingly, Tagoma et al. showed that miR-222 was increased in plasma of women with GDM compared to normoglycaemic pregnancies [76]. Moreover, Wander et al., observed no differences in the expression of miR-29a and miR-222 in the plasma of American women with or without GDM [77], thus illustrating the heterogeneity of miRNA expression.

In 2015, Zhu et al. used high-throughput sequencing and qRT-PCR to investigate miRNAs in plasma samples of Chinese women with or without GDM [78]. Five miRNAs (miR-16, miR-17, miR-19a, miR-19b and miR-20a) were significantly upregulated in women with GDM compared to controls. Furthermore, the differential expression of these miRNAs were observed at 16–19 weeks of pregnancy, before GDM diagnosis, once again illustrating the diagnostic value of miRNAs [78]. Cao et al. similarly demonstrated increased expression of plasma miR-16, miR-17 and miR-20a in a larger cohort of Chinese women, however, they did not observe differences in the expression of miR-19a and miR-19b [79]. More recently, Pheiffer et al. reported conflicting results. The expression of all five miRNAs were decreased in South African women with GDM, however, only the decreased expression of miR-20a was statistically significant [75]. Interestingly, regression analysis showed that miR-20a was a significant predictor of GDM, while age and body mass index were not.

Although these miRNAs were identified in plasma or serum, bioinformatics [75, 78] and experimental [74] functional analyses provided support for their biological relevance and role in the pathogenesis of GDM. Other studies also confirm the importance of miRNAs during GDM. For example, in 2014, Shi et al. reported that the expression of miR-222 is increased in omental tissue from women with GDM compared to women with normoglycaemic pregnancies, and conducted elegant *in vitro* experiments to demonstrate that miR-222 potentially regulates oestrogen-induced insulin resistance during GDM. As shown in **Table 1**, many more miRNAs have been reported to exhibit altered expression in maternal blood during GDM, however, these were investigated in single studies only.

6. Gestational diabetes and foetal microRNA expression

Dysregulated miRNA expression has been reported in human umbilical vein endothelial cells (HUVECs) of fetuses exposed to GDM. Floris et al. reported that

impaired HUVEC function during GDM is associated with altered miR-101 expression [80]. Several other miRNAs, miR-137 [81], miR-let-7a, miR-let-7g, miR-30c, miR-126, miR-130b, miR-148a and miR-452 [82] were upregulated in HUVECs from infants born to mothers with GDM, suggesting that miRNAs reflect the adverse *in utero* environment imposed by GDM. Tryggestad et al. further showed that two of these miRNAs, miR-130b and miR-148a, target and decrease the expression of 5' Adenosine monophosphate-activated protein kinase (AMPK α 1), whose protein expression is decreased in placenta exposed to GDM [82]. Recently, altered miRNA expression in offspring blood was shown to be associated with birth weight [83]. MiR-33b and miR-375 were overexpressed during macrosomia, while miR-454 was overexpressed in blood of both low birth weight and macrosomic compared to normal birth weight offspring [83]. Aberrant miR-346 and miR-582 expression in cord blood were shown to be associated with foetal complications [54]. Taken together, these studies provide evidence that GDM induces dysregulated miRNA expression in offspring, which may predispose them to metabolic disease in later life. Thus, miRNAs offer potential to predict disease in offspring, which could facilitate intervention strategies to prevent future disease.

7. Challenges of microRNA profiling

Despite their stability and relative ease of quantification, analysis of circulating miRNAs present several pre-analytical and analytical challenges [84] that must be addressed before they can be used clinically. Many studies have reported that miRNA expression is affected by sample type, method of miRNA extraction, and quantification and data normalisation strategies. Differences in miRNA expression between whole blood and serum [42], between different cell types in whole blood [85, 86], between plasma and serum [87, 88] and between placenta, plasma and cord blood [54] have been described. Furthermore, miRNA expression varies according to the extraction kit used [88]. Currently, qRT-PCR is considered the gold standard for miRNA analysis, however variations between qRT-PCR platforms [87] and between qRT-PCR and other measurement platforms [22, 42, 87, 88] have been widely reported. Furthermore, data normalisation is a significant challenge during miRNA profiling, particularly extracellular miRNAs [89]. Currently, there is no consensus on the best normalisation strategy to use when profiling circulating miRNAs, although strategies based on exogenous spike-in-controls such as *C. elegans* miR-39 have been shown to be less variable than using endogenous miRNAs [88]. Moreover, heterogeneous miRNA expression is observed between populations, mediated by both genetic and environmental factors [90, 91]. During pregnancy, gestation time is also reported to affect miRNA expression [55]. Lastly, miRNAs are non-specific. For example, a single miRNA can regulate up to 200 different genes [27, 28], thus miRNAs found to be associated with GDM, may possibly be involved in other conditions as well.

8. Perspectives and recommendations for future research

MiRNAs offer great potential as biomarkers for GDM. However, they face many challenges that need to be addressed before they can become clinically applicable. Standardisation of pre-analytical and analytical methods for miRNA research may minimise the lack of reproducibility between studies and should be prioritised in miRNA research [22]. MiRNAs are epigenetic mechanisms that are regulated by various factors [90, 91], which need to be considered in miRNA studies. Large

prospective cohort studies should be conducted to elucidate how biological, genetic and environmental factors affect miRNA expression, and to identify plausible diagnostic or prognostic candidates. Moreover, due to their non-specificity [27, 28], it is recommended that a panel of miRNAs, either alone, or in combination with other risk factors, should be used to increase the specificity of risk stratification models for GDM.

9. Conclusions

In this review the current status of miRNAs as biomarkers for GDM was discussed, together with recommendations for research. We provide evidence to show that miRNAs possess tremendous potential as routine screening tools, which could facilitate earlier diagnosis and management of GDM with dietary modifications or therapeutic intervention. A growing number of studies have demonstrated their clinical utility, and technological advances can lead to the development of inexpensive, point-of-care miRNA diagnostic tests in the future. However, at present miRNA profiling during GDM remains inconclusive, largely due to the irreproducibility of results between studies. Many technical, analytical and biological challenges hamper miRNA research, and must be addressed before these small RNA molecules, which are master regulators of gene expression, can become clinically applicable.

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Conflict of interest

The authors have no conflict of interest to declare.

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