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Are miR-26a and miR-26b microRNAs potent prognostic markers of gestational diabetes?

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Abstract

Background: Gestational diabetes mellitus is a common public health problem, accompanied by complications for the mother and fetus. So, introducing new biomarkers to identify early diabetes is essential. As serum miRNAs are potentially appropriate markers, we investigated miR-26a and miR-26b expression levels in pregnant women with and without gestational diabetes.

Method: Demographic and clinical characteristics of 40 gestational diabetic patients and 40 healthy controls were assessed. The expression level of miR-26a and miR-26b microRNAs was measured by real-time PCR. Statistical analysis was done with GraphPad Prism software (version 8.4.3).

Result: The findings of this study showed that the expression level of miR-26a and miR-26b increased in women with gestational diabetes compared with healthy pregnant women, but the increase in expression was only significant for miR-26a ($p < 0.05$).

Conclusion: According to the statistical and ROC curves, we suggest miR-26a as a potential biomarker for the early diagnosis of gestational diabetes mellitus.

KEYWORDS

biomarker, diagnosis, gestational diabetes, miR-26a, miR-26b, prognosis

1 | INTRODUCTION

Every year, many people die due to diabetes or its complications.[1] Diabetes mellitus is one of the leading causes of death; currently, at least 400 million people worldwide suffer from this disease.[2] The diabetes prevalence in people between 20 and 79 years old all over the world in 2021 was reported to be around 10.5%, with a sex-independent prevalence and highest in the age group of 75–79 years. The associated health expenditures of this global problem were estimated at 966 billion USD (United States Dollar) in 2021.[2] Based on pancreas defects, diabetes is divided into two types: type 1 diabetes (insulin-dependent) (IDDM) and type 2 diabetes (noninsulin-dependent) (NIDDM). Diabetes is not limited to the abnormality of blood glucose levels; if it is not controlled, it also affects other body systems.[3,4] One of the NIDDM kinds is gestational diabetes mellitus (GDM).[5,6] GDM is an increasingly common metabolic disorder during pregnancy due to obesity, inactivity, and the aging of mothers and endocrine disrupting chemicals (EDCs).[5–7] EDCs, which are compounds found in food preservatives, medical care products, personal care products, and homes, include phenol, phthalates, and parabens. Studies have revealed that these compounds are linked with diabetes.[7,8] GDM starts and is diagnosed for the first time during

pregnancy in different severity of glucose intolerance.[9,10] GDM can be diagnosed in the second trimester and at the beginning of the third trimester.[9] However, it increases the risk of developing type 2 diabetes during life.[5]

The prevalence of gestational diabetes varies among different studies based on the screening method and criteria used.[11] By 2020, the prevalence of GDM was 14%. Before this year, the prevalence of GDM varied across different regions of the world. The Middle East and North Africa had the highest prevalence at 27.6%, while South-East Asia had a prevalence of 20.8%. Other continents had varying prevalence rates, with the Western Pacific at 14.7%, Africa at 14.2%, South and Central America at 10.4%, Europe at 7.8%, and North America and the Caribbean at 7.1%.[11] It is worth noting that the prevalence of GDM has increased in high-income countries, reaching 14.2%.[11] GDM can have irreparable consequences for the mother and fetus.[12] For example, elevated maternal blood glucose levels in gestational diabetes increase fetal glucose and, as a result, increase insulin secretion in the fetal body.[6,13,14] This upregulates the fetal growth factors, which cause complications such as macrosomia of the fetus, premature birth, and early fetal damage.[6,15,16]

The pathology of GDM is still not fully understood. Inadequate adaptation of pancreatic beta cells to insulin resistance in the second and third trimesters of pregnancy can be one of the leading causes of GDM, while in normal pregnancy, glucose balance is regulated by increasing insulin secretion.[17] Some risk factors for gestational diabetes include overweight, waist fat, hereditary background, and a history of gestational diabetes.[18–22] Due to the life-threatening complications of diabetes, early and accurate diagnosis, prognosis, and treatment are essential. Till now, several biomarkers have been studied to identify diabetes[23]; among them, serum miRNAs are considered potentially appropriate markers because of their abundance, stability, and potential relation to specific diseases.[24,25] MicroRNAs (miRNAs) are small noncoding RNAs that bind directly to the end of 3′-UTRs and, by creating DNA/RNA hybrids, degrade mRNA and inhibit translation.[26] One of the evaluated miRNAs in diabetes is the miR-26 family, which is a group of conserved miRNAs, including miR-26a, miR-26b, miR-4465, and miR-1297 with the same sequence "UCAAGUA" among vertebrates.[27] miR-26a-1, miR-26a-2, and miR-26b are located on chromosomes 3, 12, and 2, respectively.[28,29] miR-26a-1 and miR-26a-2 share the same sequence and differ from miR-26b in two nucleotides.[28] It has been found that miR-26 plays an essential role in the growth, development, and cell differentiation of different tissues.[27,28] miR-26a has an inverse correlation with the sucrase-isomaltase (SIE) enzyme, and high glucose concentrations stimulate the transcription of this miR-26a.[30] Also, miR-26a enhances brown fat, miR-26a was found to significantly alter mitochondrial morphology toward that seen in brown adipocytes, thus inducing pathways related to energy wastage.[31] Two key regulators of adipocyte differentiation, miR-26a, and miR-26b, are required for adipogenesis and play an important role in determining the characteristics of brown fat cells that produce heat.[31] These miRNAs induce thermogenesis in BAT or WAT and increasing energy consumption, which in turn prevents the development of obesity caused by diet and metabolic dysfunction, as well as complications of obesity, including diabetes.[31–33]

Studies have shown that a set of miRNAs regulates insulin synthesis. Deactivating some miRNAs, such as miR-26a, miR-182, and miR-148, in the culture medium of pancreatic beta cells reduces insulin promoter activity and insulin mRNA levels.[32] miR-26a targets factors encoding insulin cell signals (Glycogen synthase kinase-3 beta (GSK3β), Protein kinase C δ (PKCδ), Protein kinase C θ (PKCθ), fatty acid metabolism (ACSL3 (Acyl-CoA synthetase long chain family member 3), ACSL4 (Acyl-CoA synthetase long chain family member 4)), and gluconeogenesis. miR-26a underexpression decreases insulin receptor gene promoter activity, receptor mRNA levels, and insulin receptors. So, it leads to insulin resistance and type 2 diabetes.[34]

The miR-26a overexpression significantly leads to serine amino acid phosphorylation of AKT in the insulin signaling pathway, which causes GLUT4 (Glucose transporter type 4) to be sent to the cell surface to receive glucose[35] (Figure 1). miR-26b plays a crucial role in cell growth and development, especially in tumors, but it is also an essential mediator in nontumor disorders such as gestational diabetes.[36,37] The inflammation mechanism in GDM involves hypoxia-inducible factor (HIF)−1, which, as an extracellular glycoprotein, adjusts downstream agents. With HIF-1 blockage, the PI3K/Akt (phosphatidylinositol 3-kinase/protein kinase B) signaling pathway will be inhibited. Therefore, the miR-26b inhibition leads to HIF-1 promotion and relieves the inflammation symptoms in GDM[38] (Figure 1).

Considering that prediabetes is a step before developing diabetes mellitus without all the diabetes symptoms and abnormal blood sugar, it is not diagnosed accurately. Indeed, this condition is not considered a clinical disorder, but it can be a risk for developing diabetes and its life-threatening conditions, such as cardiovascular diseases. Considering the importance of early diagnosis of gestational diabetes and the prevention of its complications for mother and fetus, our aim in this study is to investigate the expression level of microRNAs miR26a and miR26b as possible biomarkers for early diagnosis of gestational diabetes.

2 | MATERIALS AND METHODS

2.1 | Participants and ethical considerations

The study subjects were pregnant women for the first time and had no history of diabetes. These women visited health centers in Ilam City for routine blood sugar checks. Among these people, 40 people with diabetes without a history of other diseases and 40 healthy pregnant women were randomly included in the study. Participants' blood samples were collected between 24 and 28 weeks of pregnancy to measure biochemical factors and the expression level of microRNAs miR26a and miR26b. The necessity of the confidentiality of the participants' information and the freedom to participate in this research were explained to all of them. Then, all participants signed the consent forms. The study was performed under the ethical code of IR.MEDILAM.REC.1397.158.

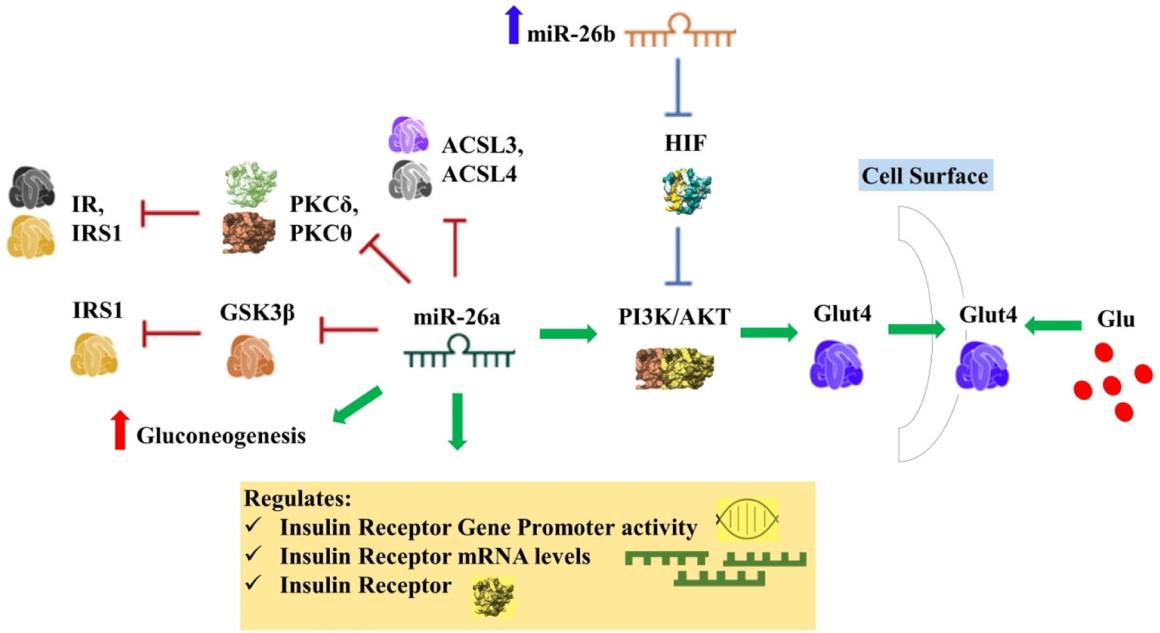

FIGURE 1 Mechanisms of actions of miR-26 family.

2.2 | Sample collection and RNA extraction

Five ml of peripheral blood was collected from all people in EDTA (ethylenediaminetetraacetic acid) tubes. Subsequently, the blood samples were centrifuged at 3500 rpm for 10 min to detach the serum. Total RNA was extracted from the serum using the RNX kit (EX6101; Cinnagen) based on the manufacturer's instructions. The obtained RNA was evaluated qualitatively and quantitatively by gel electrophoresis and the spectrophotometer. DNA contamination was eliminated according to the DNaseI (Fermentas, Lithuania) protocol.

2.3 | cDNA synthesis and real-time PCR assay

According to the manufacturer's rules, cDNA was synthesized by 3 µg of pure total RNA and High-Capacity cDNA Reverse Transcription Kits (PN: 4375575; Applied Biosystems). The miR-26a and miR-26b expressions were reported and compared with Snord47 (Small Nucleolar RNA, C/D Box 47) as an internal control gene using relevant specific primers (Table 1). Real-time PCR was performed in the ABI 7500 system (Applied Biosystems) using 10 µL of BIOFACT™ 2X Real-Time PCR Master Mix, 8 ng cDNA, and 250 nM of each primer twice. ΔCT means for all participants were measured, and ultimately, the fold changes of miR-26 a and b expressions were calculated by ratio = $2^{-\Delta\Delta C_t}$ based on Livak suggestion.[39]

2.4 | Statistical analysis

Statistical analysis was performed by GraphPad Prism software (version 8.4.3). Based on the Kolmogorov–Smirnov test, our data was normal. Student's *t* test was applied to compare the expression difference of every microRNA between GDM patients and healthy controls. Pearson's correlation test was used to evaluate the relationship between the expression of different miR-26a and miR-26b. The specificity and sensitivity of miR-26a and miR-26b as potential biomarkers for diagnosing GDM were assessed by receiver operating characteristic (ROC) curve.[40] Error values less than 0.05 were defined as significant differences.

TABLE 1 Primers used in RT-qPCR.

Gene primers	Primer	Primer sequences
miR-26a	Forward	AGT ATT CCT GGA TAG GC
miR-26b	Forward	AGT TCA AGT AAT TCA GGA TA
Snord47	Forward	ATC ACT GTA AAA CCG TTC
Common	Reverse	GAG CAG GGT CCG AG

3 | RESULTS

3.1 | Cases and controls and serum biochemical tests

The demographic and biochemical parameters of 40 GDM patients and 40 controls who participated in this study are shown in Table 2. In this study, the biochemical factors FBS, LDL, TG, and cholesterol showed a significant increase in GDM women compared with healthy pregnant women. However, there was no significant difference in HDL levels in the group.

3.2 | miR-26a and miR-26b expression patterns in GDM and healthy individuals

Based on our tests, the levels of miR-26a and miR-26b expression were higher in women with GDM compared with healthy pregnant women. However, only miR-26a expression was significantly higher ($p < 0.05$) (Figure 2).

TABLE 2 Demographic and biochemical parameters of participants.

Variable	GDM group ($n = 40$)	Control group ($n = 40$)	p Value
Age (year)	3.3 ± 28.6	4.4 ± 26.3	–
Gestational age	4.4 ± 22.5	5.1 ± 22.3	–
Systolic blood pressure (mg/dL)	13.71 ± 124.33	7.55 ± 118.47	$p > 0.05$
Diastolic blood pressure (mg/dL)	4.36 ± 82.22	4.40 ± 89.81	$p > 0.05$
Fasting blood sugar (mg/dL)	22.5 ± 139.50	13.97 ± 78.52	$p < 0.01$
Total cholesterol (mg/dL)	25.06 ± 173.36	23.10 ± 167.35	$p > 0.05$
HDL (mg/dL)	11.07 ± 46.02	13.40 ± 49.08	$p > 0.05$
LDL (mg/dL)	32.01 ± 131.47	20.08 ± 126.47	$p > 0.05$
Triglyceride	3.3 ± 203.7	4.46 ± 172.7	$p < 0.05$

3.3 | The specificity and sensitivity of target genes as potential biomarkers

ROC curves showed the specificity and sensitivity of miR-26a with an AUC (Area under the ROC curve) of 0.63 ($p < 0.05$), and miR-26b with an AUC of 0.56, respectively (Figure 3).

4 | DISCUSSION

Many pregnant women worldwide have gestational diabetes, as the most common metabolic disorder in this era. The remarkable point is that its rapid spread in the last decade is fully related to maternal obesity.[12,41–44] GDM is traditionally defined as any grade of glucose intolerance during pregnancy.[45,46]

This disease is characterized by the inability of beta cells to respond to the increased need for insulin during pregnancy, resulting in different blood sugar levels.[47] Improper blood sugar control in diabetic pregnant women leads to adverse effects on the fetus throughout the pregnancy. In the first trimester of pregnancy, maternal hyperglycemia can cause diabetic embryopathy, which leads to severe congenital disabilities and even spontaneous abortion.[6,13,48,49] The specific mechanisms by which hyperglycemia impairs the development of the fetus are not fully understood, but reduced arachidonic acid and myoinositol and the accumulation of sorbitol and trace metals in the fetus in animal models have been reported as the causes of this phenomenon.[48,50–52] To diagnose and control diseases like the metabolic disorder of diabetes, several biomarkers have been investigated. Blood microRNAs are considered

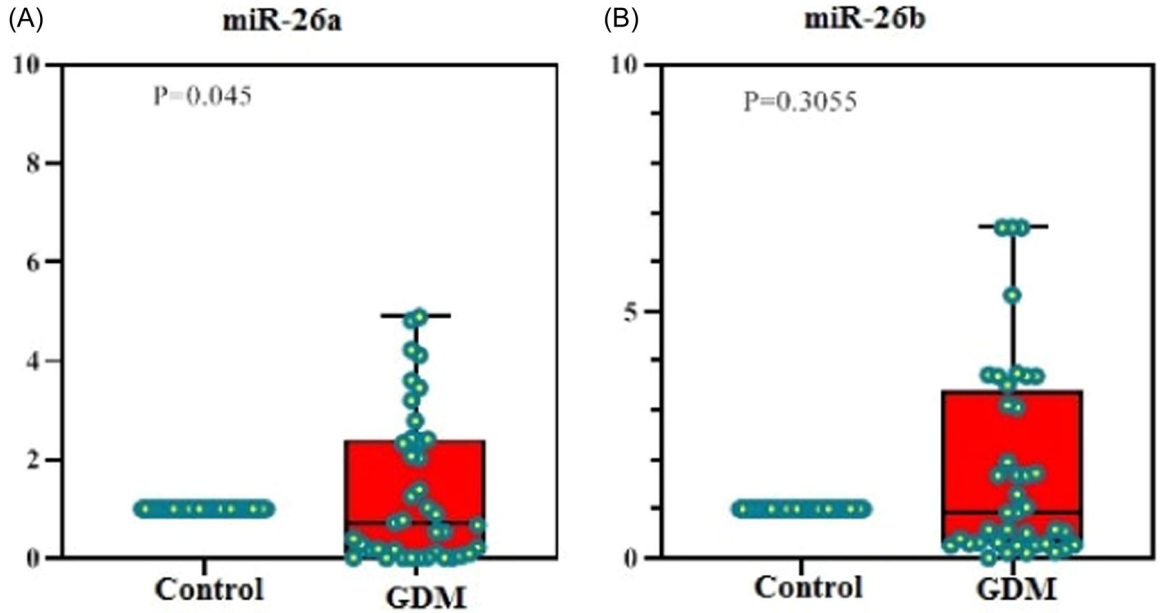

FIGURE 2 Expression pattern of miR-26a/Snord47 (A) and miR-26b/Snord47 (B) relavive expression in the serum of GDM and control people. The expression levels of miR-26a and miR-26b increased in women with gestational diabetes compared with healthy pregnant women. The increase in expression was only significant for miR-26a ($p < 0.05$).

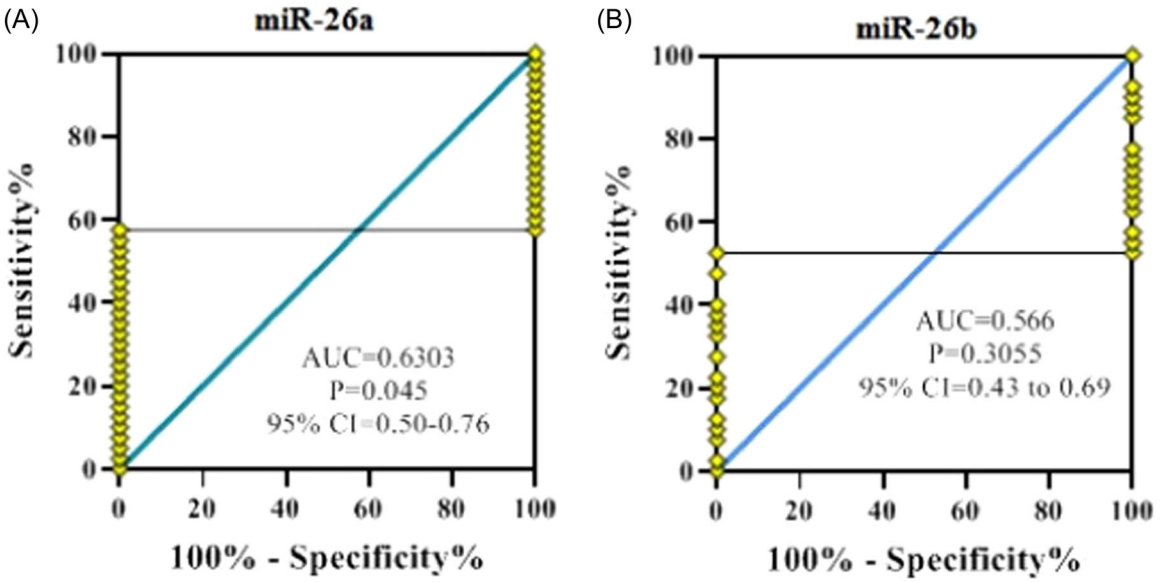

FIGURE 3 Receiver operating characteristic (ROC) curve of miR-26a (A) and miR-26b (B).

potential biomarkers because of their numerous advantages, such as their abundance and stability in serum and their potential relationship to certain diseases. So, using serum microRNAs as biomarkers is preferable.[24,25,53]

The discovery of miRNA is one of the most important scientific advances in recent years, which has revolutionized molecular biology and medical sciences.[53,54] MicroRNAs can generally change the genomic pattern of many biological processes.[55] The literature showed a relationship between the expression of microRNAs in pregnant women with insulin resistance, which confirms the claim that miRNAs can be considered diagnostic or therapeutic biomarkers of gestational diabetes.[56] The present study investigated miR-26a and miR-26b expression levels in pregnant women with diabetes and healthy pregnant people. The results indicated that the expression level of miR-26a and miR-26b in pregnant women with diabetes was increased compared with the control group, but this elevation was significant only in miR-26a.

Based on real-time PCR and ROC curve analyses, we can introduce miR-26a as a potential biomarker for the diagnosis of GDM. In studies conducted on the expression of miRNAs as biomarkers in gestational diabetes, miR-195-5P,[57] miR-20a-5p, miR-16-5p, and miR-17-5p,[58,59] showed significant overexpression. Also, the expression of miR-33a-5p was increased in pregnant women with diabetes. miR-33a affects INS-1 (Gene encoding insulin 1) and insulin production by targeting ABCA1 (ATP-binding cassette transporter).[60] But in GDM, some miRNAs like miR-222, miR-132, miR-29a, miR-30d, miR-96, and miR-185 were underexpressed.[24,61–64] Animal studies showed that the increase of miR-26a through the insulin signaling pathway is involved in the increase of glucose absorption. A study on diabetic mice showed that miR-26a improves insulin resistance and glucose balance and increases the quality of bone formation, and administration of miR-26a to diabetic mice promotes phosphorylation of downstream mediators in the insulin signaling pathway. In addition, diabetic mice have fewer insulin receptors than healthy mice, and miR-26a increases these receptors.[35] Another study showed that miR-26a downregulation in the exosome of overweight and obese individuals and the increase of this miRNA improve glucose homeostasis and insulin sensitivity. This study also showed that miR-26a reduces beta-cell hyperplasia by reducing cell division and inhibiting high insulin secretion in response to hyperglycemia.[65]

5 | CONCLUSION

The results of the present study indicate that with the increase in blood sugar levels in pregnant people, the miR-26a expression also increased significantly, which can be caused by the compensatory mechanism of miR-26a, which affects the pathway of increased activity of GLUT4 and increased expression of the insulin receptor, which can increase maternal blood glucose absorption. Considering the increased expression of miR-26a in pregnant women with gestational diabetes compared with healthy pregnant women, as well as the compensatory mechanism of miR-26a in the regulation of increased sugar in diabetic conditions, it may be possible to use miR-26a in early diagnosis, prognosis, and even treatment in the future. More investigation into the expression pattern and mechanism of action of the mir-26 family is highly recommended for GDM.

AUTHOR CONTRIBUTIONS

Hori Ghaneialvar designed the study. Hori Ghaneialvar, Saeed Kakaee, Azra Kenarkoohi, and Mahdieh Mehrab Mohseni performed the experimental investigation. Hori Ghaneialvar and Mahdieh Mehrab Mohseni analyzed the data. Hori Ghaneialvar and Mahdieh

Mehrab Mohseni wrote the paper. All the authors have read and contributed to the final manuscript and approved its submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All of the data is available.

ETHICS STATEMENT

Human participation in this study was under the ethical standards of the National Research Committee and the 1964 Helsinki Declaration and its later amendments. All participants were individually informed that their data should be used for scientific research, and then they provided informed consent. This study was approved in advance by the Ethics Committee of Ilam Medical Sciences University (ethics number: IR.MEDILAM.REC.1397.158).

TRANSPARENCY STATEMENT

The lead author Hori Ghaneialvar, Saeed Kakaee affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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