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Do the expression of long noncoding RNA MALAT1 and inflammatory factors help diagnose gestational diabetes in the second trimester of pregnancy?

Mojdeh Ghiyas Tabari[1, 6], Farzin Sadeghi[2, 5], Mahmood Hajiahmadi[3], Shahnaz Barat[4], Hadi Parsian[1, 5*]

[1]Department of Clinical Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, **Iran**
[2]Department of Virology, School of Medicine, Babol University of Medical Sciences, Babol, **Iran**
[3]Department of Biostatistics, Faculty of Medicine, Babol University of Medical Sciences, Babol, I**ran**
[4]Department of Obstetrics and Gynecology, Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, **Iran**
[5]Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, **Iran**
[6]Student Research Committee, Babol University of Medical Sciences, Babol, **Iran**

***Correspondence:** hadiparsian@mubabol.ac.ir **Received 20-12-2021, Revised: 14-04-2022, Accepted: 20-04-2022 e-Published: 27-04-2022**

Gestational diabetes mellitus (GDM) is a common clinical disease characterized by intolerance to glucose and varying its severity in the second or third trimester of pregnancy. Inflammatory mediators such as IL-6 and TNFα are thought to contribute to the development of gestational diabetes. In addition, various long noncoding RNAs (LncRNAs) such as MALAT1 are suggested as epigenetic modifiers using chromatin-modifying complexes at specific genomic sites or promoter regions. Today, researchers are paying close attention to understand the role of LncRNAs expression status in various diseases. The study was designed to analyze the association of inflammatory mediators IL6, TNFα, and LncRNA MALAT1 expression status with the development of GDM in the second trimester of pregnancy. In this case-control study, 30 pregnant women diagnosed with GDM and 30 pregnant women having normal glucose tolerance tests participated. All participants were screened according to the criteria of the ADA. Expression levels of IL-6, TNFα, and MALAT1 were analyzed by q RT-PCR. The results were analyzed statistically by SPSS 22 software. There was a significant difference in the levels of IL6 (p value=0.049), but not MALAT1 and TNFα (p= 0.42 and p=0.79, respectively) in the two groups. The results of ROC analysis for discriminating of patients with GDM and healthy individuals were only reasonable for IL6 (AUC=0.771, sensitivity = 0.867, specificity = 0.733, PPV%=87, NPV%=73).Although the basic diagnosis of gestational diabetes relies on a glucose test, finding new diagnostic factors is also necessary. According to the results, it seems that IL6 expression status, along with blood glucose, can be used as diagnostic biomarkers in the diagnosis of GDM in the second trimester of pregnancy. More studies are needed for the determination of the exact role of IL6 in the pathogenesis of GDM.

Keywords: Long non-coding RNA, TNFα, Interleukin-6, Gestational diabetes mellitus.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a disorder of glucose tolerance that occurs with varying severity during pregnancy (Baz B et al.2016). During the first trimester of pregnancy, a pregnant woman is unable to secrete enough insulin due to changes in nutritional increase and elevated production of fat, reduced insulin sensitivity, and anti-insulin hormones, such as human placental hormone, prolactin, cortisol, estrogen, and progesterone produced during pregnancy (Chen X et al.2013). The state of insulin resistance peaks in the third trimester of pregnancy (Dennedy MC et al.2010). In communities where type 2 diabetes is more prevalent, gestational diabetes is also more common, but the time of onset and the extent of its effects vary widely(Kim C.2013). It is estimated that GDM recurs in 30 to 70% of subsequent pregnancies (Lindsay KL et al.2020). GDM is linked to the progressing incidence of adverse effects for both fetal and maternal, including congenital malformations, macrosomia, spontaneous abortion, preeclampsia, and perinatal mortality (McIntyre HD et al.2019). The blood glucose test is performed at 24-28 weeks of pregnancy for GDM diagnosis, along with an oral glucose tolerance test (GTT). There are several criteria for diagnosing GDM (Mpondo BC et al.2015). Based on the widely used diagnostic criteria as outlined in ADA guidelines, more pregnant women are diagnosed

with gestational diabetes than before (Osgood ND et al.2011). Most women with gestational diabetes return to normal glycemia after giving birth. However, these patients are more likely to develop type 2 diabetes in the future (Ryan E.2011). Hence, it is important to find a suitable and early diagnostic method to prevent complications for the mother and baby. One of the diagnostic methods that have recently been considered is studying the expression status of LncRNAs.

In animal cells, the bulk of genomic DNA transcripts arise from non-coding RNAs (ncRNAs) that do not encode any proteins (Zhu Y et al.2016). The ncRNAs that have more than 200 nucleotides and are not capable of protein-synthesizing are called long non-coding RNAs (LncRNA) (Ghayor C et al.2016). Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) is a well-studied LncRNA with a length of about 8.7 kb. It contains two exons and is located on the human chromosome 11 q13.1 [12]. The MALAT1 gene does not have open reading frames and cannot translate into a protein (Yoshimoto R et al.2016). MALAT1 is located in the nuclear speckle region and can be detected in many tissues and bodily fluids. In recent years, several studies have demonstrated its alterations in multiple disease processes (Gutschner T et al.2013).

It seems that during GDM, changes in multiple inflammatory cytokines occur, which may subsequently cause tissue damage (Plows JF et al.2018). Advanced glycation end products produced as a result of hyperglycemia increase oxidative stress factors (Desoye G.2007). They can activate macrophages and increase serum levels of proinflammatory cytokines such as IL6 and TNFα. These proinflammatory cytokines may play a critical role by further destroying pancreatic beta cells and augmenting hyperglycemia (Lappas M et al. 2010, De Luca C et al.2008). Recently, an association between MALAT1 and inflammatory factors involved in gestational diabetes has been demonstrated. Zhang et al. observed an increase in serum MALAT1 levels in gestational diabetes patients (Zhang Y et al.2018). The regulatory role of MALAT1 was also demonstrated in another study using cultured tissue from the placenta of mothers with gestational diabetes and healthy mothers (Zhang Y et al.2020). According to our knowledge, no study assesses the status of these molecules in the second semester of pregnancy. Therefore, we aimed to evaluate the expression of MALAT1 in GDM patients and its possible relationships with inflammatory factors, like IL6 and TNF α at the second trimester of pregnancy.

MATERIALS AND METHODS

This study, 30 patients diagnosed with GDM (mean age 25.7±4.4 years old) and 30 healthy pregnant women as the control group were participated (mean age 24.8±4.4 years old). Pregnant women in the second trimester of pregnancy (24-28 weeks) were referred to one of our university-affiliated hospitals. Maternal height and weight were measured and body mass index (BMI) was calculated. Patients with a history of hemolytic anemia, hemoglobinopathy, kidney disease, previous diabetes, and infectious diseases were excluded. All participants completed detailed questionnaires regarding their medical history and socio-demographic characteristics. Informed consent was obtained from all individuals.

Blood was collected from all participants following a standardized protocol with 8-12 hours of fasting. GDM was diagnosed when the serum glucose levels were abnormal (according to the ADA guidelines)(Goyal A et al.2020), which indicated levels ≥92, 180, and 155 mg/dl for fasting, 1-hour and 2-hour for OGTT, respectively, after receiving 75 g glucose. One or more abnormal glucose levels resulted in a GDM diagnosis. The venous plasma glucose levels were measured using the glucose oxidase method (Pars Azmon Kit) (Burtis CA et al.2012), with a Hitachi 7180 Automatic Analyzer (Hitachi Co, Tokyo, Japan).

RNA isolation

Two ml of whole blood was used for RNA extraction and PBMCs were isolated using a phycol gradient centrifuge. RNA extractions were performed using a Super RNA extraction kit and following the manufacturer's protocol (Yekta Tajhiz Company, Iran). RNA purity and concentration were assessed by NanoDrop (Boeco, Germany). The resulted RNAs were stored at −70°C until final analysis.

After RNA isolation and quality assurance, cDNA synthesis steps were done.Reverse transcriptions were carried out using Easy Script First-strand cDNA synthesis supermix (Yekta Tajhiz Company, Iran) following the manufacturer's instructions. Gene-specific primer sets were designed using the BLAST from NCBI and were provided by Metabion International AG (Germany).

Table1: Primer sequences for quantitative real-time PCR

Gene	Reverse(5′-3′)	Forward(5′-3′)
GAPDH	CCCTTTTGGCTCCACCCT	TTCACCACCATGGAGAAGGC
IL-6	CCATCTTTGGAAGGTTCAGGTTG	ACTCACCTCTTCAGAACGAATTG
TNFα	CGGGCCGATTGATCTCAGC	GAGGCCAAGCCCTGGTATG
MALAT1	ATTCGGGGCTCTGTAGTCCT	GACGGAGGTTGAGATGAAGC

cDNA synthesis

First,1 µl random primers, 6-8 µl RNA, 1 µl Oligo dT, and nuclease-free water were mixed and incubated at 65°C for 5 minutes (to bind the primer). Then the tubes were placed on ice for 2 minutes and then the rest of the materials (10 µl reaction mix and 1 µl enzyme mix) were added (for better efficiency as recommended by the kit). The final volume, by adding free nuclear water, was 20 µl.

It was then incubated at 42°C for 30 minutes and then incubated for 5 seconds to inactivate the enzyme at 85°C.

Quantitative real-time polymerase chain reaction

Quantitative evaluation of gene expression with synthesized cDNA was performed by Real-time PCR, SYBR Green method. All real-time PCR steps were performed according to the PCR Master Bio Sy Green mix (Biosystem) with polymerase activation at 95°C for 30 seconds, denaturation at 95°C for 5 seconds, annealing for TNFα, IL6, and MALAT1 at 62°C, 61°C, and 60°C, respectively, followed by 40 cycles for each of them and finally Extension, at 720C for 30 seconds.

At the end of the cycle, to confirm the correct amplification and specificity of the genes, their melting curve was examined. The melting curve was plotted by the Q Rotorgene and the raw data were extracted as Ct. Rotorgene 6000 program was used to analyze them. After obtaining Ct of the studied genes in all samples, the related ΔCt (the result of subtracting the Ct of the studied genes from the Ct of the internal control gene, i.e., GAPDH) were calculated. In this study, the pfaffl method was used to analyze the data obtained from Real-Time PCR (Pfaffl MW et al.2001).

Statistical analysis

The results were analyzed statistically using SPSS 22 software. Normal distributions of the variables were assessed by the Kolmogorov-Smirnov test. T-Student tests were used for the evaluation of significant differences between the two groups. Statistical significance was defined as P-value less than 0.05. A simple linear regression test was used to determine the effect of variables. Pearson correlation analysis was also used to examine the correlation between variables. The ROC curve was analyzed to evaluate the diagnostic accuracy of the evaluated factors for discriminating patients with gestational diabetes over healthy individual

Sixty pregnant women entered the study according to their laboratory data and were diagnosed by a gynecologist, 30healthy pregnant women as a control group, and 30 patients with GDM.

GDM was diagnosed according to the ADA 2020 (Gestational Diagnosis Protocol with 75 grams of oral glucose). The reference range for GTT1hr was considered lower than 180 mg/dl and GTT2hr was considered lower than 153 mg/dl. The mean of GTT1hr in the healthy group was 154 mg/dl and in the GDM group was 188.8 mg/dl (P <0.001). Similarly, GTT2hr was 148.5 mg/dl in the healthy group and the GDM group was 178.1 mg/dl (P <0.001). The FBS levels were also significantly lower in the control group compared to the GDM group (P <0.001) (see Table 2).

As shown in Table 3, the expression of IL6 in the control group was significantly lower than in the GDM group (P=0.049). However, the expression status of TNFα and MALAT1 levels were not different between the control and GDM groups (P = 0.79 and 0.42, respectively), although a trend of an increase in crude data (not significant) was observed for MALAT1.

By using simple linear regression analysis, no significant relationship between MALAT1 and TNFα (P-value=0.11, r=0.29, CI95%: 0.14-1.31, SD=0.35 and B=0.58) and between MALAT1 and IL6 (P-value=0.19, r=0.24, CI95%- .08-0.23, SD=0.32 and, B=-0.42) were observed. Furthermore, no significant relationships were observed between FBS and MALAT1(r= -0.28, P-value=0.12), TNFα (r= -0.07, P-value=0.69) and IL6(r= -0.07, P-value=0.7).

Roc curve analyses were done for all included parameters for discrimination of GDM and healthy groups. Only the results were reasonable for IL6. The cutoff value of 1.79 IL6 fold changes showed the best diagnostic accuracy for discriminating the GDM patients than healthy subjects; (see Figure 1). The details of IL6 diagnostic accuracy are presented (see Table 4).

RESULTS

Table2: Clinical and demographic characteristics of the included groups

Groups	Age (Year)	BMI (Kg/m^2)	FBS (mg/dl)	GTT1 (mg/dl)	GTT2 (mg/dl)
Control	4.4±24.8	1.4±25.3	79.7±8.7	13.9±154	12.3±148.5
GDM	25.7±4. 4	26.6±1.2	102.5±4.	188.8±12.3	178.1±12.7
P value	0.53		<0.001	0.004	<0.001

SD± results are reported as mean

Table: 3 Comparison of gene expression levels in included groups

Groups	MALAT1 (FC relative to IS)	IL6 (FC relative to IS)	TNFα (FC relative to IS)
Control	1.3±1.1	2.5±3.4	3.7±5.9
GDM	1.8±1.9	4.7±3.8	3.5±3.8
P value	0.42	0.049	0.79

†Fold change (FC) relative to internal standard (IS)

Table 4: Diagnostic value of IL6 for discrimination of GDM patients than healthy individuals in Trimester2

AUC	Cut off	Sensitivity	Specificity	PPV%	NPV%
0.771	1.79	%86.7 % CI=0.75- 0.99	%73.3 % CI =0.51- 0.96	%87 % CI=75- 99	%73 % CI=51- 96

% CI =Confidence interval %95
PPV%=Positive predictive value
NPV%=Negative predictive value

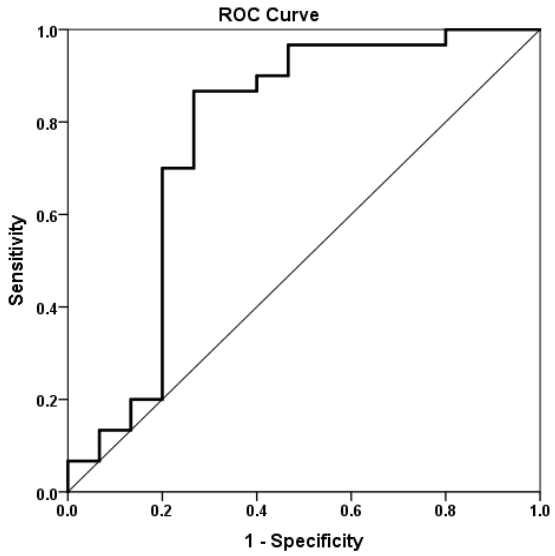

Figure1: Potential diagnostic accuracy of IL6 for discriminating of GDM patients than healthy controls (AUC=0.771, cut off= 1.79, % CI= 0.59-0.94)

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DISCUSSION

GDM is usually diagnosed between 24-28 weeks of gestation (McIntyre HD et al 2019, Chiefari E et al.2017). Recent research suggests that on average, one in seven births is associated with maternal GDM (Tumurbaatar B et al.2017). However, the incidence range varies between 1-14% in various populations. During pregnancy, the body naturally becomes resistant to insulin. If insulin resistance becomes severe, it may lead to gestational diabetes, which can have both short-term and long-term complications for both the mother and fetus (Zhu Y et al 2019, Mack LR et al.2017). The exact cause of gestational diabetes has not been identified (Plows JF et al.2018). Alterations of inflammatory cytokines such as TNFα and IL6 and their regulation by LncRNAs have been recently gained attention in this area. Long ncRNAs can regulate gene expression at various epigenetic levels, modulating transcription and post-transcriptional processes. MALAT1 regulates gene expression by interacting with the polycomb2 inhibitory complex (PRC2) (Sun YY et al.2020). MALAT1 interacts with RNA polymerase 2 and transcription factors to regulate transcription. In a normal pregnancy, cytokines in the mother's serum are not detectable. Increasing the level of inflammatory cytokines in the mother's serum can cause an inflammatory cascade in the mother's body and cause problems for both the mother and fetus (Chang C-P et al.2016).

In this study, no significant difference in TNFα and MALAT1 gene expression was observed between the two groups. Various studies have shown differential expression of these two genes in GDM and diseases caused by complications of diabetes. However, we found that IL6 was elevated in the GDM group. ROC analysis showed that IL6 with an AUC of 0.771 (cut-off point of 1.79 FC) and a sensitivity of 86.7% and a specificity of 73.3% can be a possible diagnostic criterion for discrimination between patients with gestational diabetes than healthy ones.

Contrary to our results, Zhang et al. observed an increase in serum MALAT1 levels in the gestational diabetes group in the third trimester of pregnancy (36-40

weeks) (Zhang Y et al.2018). Moreover, in another study, it was demonstrated a high expression level of MALAT1 by using cultured tissue from the placenta of GDM patients and healthy ones (Zhang Y et al.2020). In other organs affected by diabetic complications, RNA microarray profiling has shown an increase in MALAT1 expression in the retina of diabetic mice and retinal endothelial cells exposed to high levels of glucose (Chen H et al.2018). In further studies, both in vivo and in vitro models of diabetic retinopathy, it has been shown an upregulation of the MALAT1 gene and an increase in inflammatory cytokines (IL6 and TNFα) (Zhou L-j et al.2020).

In another study by Biswas, S et al., which was performed on a model of diabetic retinopathy (HRECs cells at high glucose concentrations), along with upregulation of MALAT1 gene and an increase in inflammatory cytokines (IL6 and TNFα), elevated expression of PRC2 were also reported (Biswas S et al.2018). Gorden et al. showed an increase in inflammatory cytokines such as IL6 and TNFα along with upregulation of the MALAT1 gene in human umbilical vein endothelial cells (HUVECs) (Gordon AD et al.2018). Wang et al. reported an upregulation of the MALAT1 gene along with an increase in inflammatory cytokines such as IL6 and TNFα (a cellular model of diabetic retinopathy at high glucose concentrations) (Wang Y et al.2020). In all studies performed on patients with diabetes-related complications, as well as a few limited studies of gestational diabetes, upregulation of MALAT1 gene along with increased IL6 and TNFα gene expression (in a diabetic cell line model and diabetic animal models) was reported (Abdulle LE et al.2019). However, in this study, no significant difference in TNFα and MALAT1 gene expression was observed between the two groups. In addition, a trend of an increase in crude data (not significant) was observed for MALAT1. Several reasons may be responsible for this, including small sample size, investigation in the second trimester as well as population-specific alterations.

It is important to note that we found a significant increase in the gene expression levels of IL6, similar to other studies. In numerous studies, the IL6 level was significantly higher in the second trimester of pregnancy in GDM patients compared to the control group (MORISSET AS et al 2011 ; Zhao X et al.2018; Zhang Jet al.2017; Amirian A et al.2020; Gomes CP et al.2013; Al-Musharaf S et al.2021; Kuzmicki M et al.2009). While in some other studies in the second trimester of pregnancy, no significant difference was observed (Braga FO et al.2019; Özyer Ş et al.2014; Pöyhönen-Alho M et al.2011; Yu H et al.2018).

IL6 is not only an intermediate molecule in inflammation but also is considered a cause of type 1 and type 2 diabetes. It is involved in the regulation of homeostasis and glucose metabolism in the activity of skeletal muscle cells, adipocytes, hepatocytes as well as pancreatic beta cells (Kim JH et al.2009). In GDM, inflammatory cytokines may cause insulin resistance by suppressing various pathways that are responsible for appropriate insulin signaling (Nergiz S et al.2014). The JAK-STAT pathway is a way of transmitting external signals like IL6 into the cell, in which case the activity of the SOCS family gene increases. The JAK / STAT pathway activates the SOCS family (insulin signaling suppressor proteins). SOCS can inactivate IRS-1 and cause insulin resistance. In addition to the JAK/STAT signaling pathway, other studies suggested pathways such as MAPK, PI3K, P38, and Jnk are also involved (Dodington DW, Desai HR.2018). The exact mechanism of these pathways is not well understood. Surely, further detailed analysis is needed to address the exact mechanism.

CONCLUSION

The results of this study showed that IL6 expression is increased in GDM, which suggests that changes in blood glucose levels can alter IL6 expression. In addition, the high significant value of IL6 expression in GDM seems to be an effective diagnostic tool. It seems that the sensitivity of 86.7% and a specificity of 73.3% are having an appropriate diagnostic accuracy to diagnose healthy individuals than GDM subjects. Probably analysis of IL6 can be used to screen, predict, and diagnose gestational diabetes.

Ethics approval

All participants signed written consent. The written consents were approved by the ethical committee at Babol University of Medical Sciences. The code of ethics committee was IR- MUBABOL. HRI.REC.1398.160.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

This article is taken from a PhD student dissertation. Mojdeh Ghiyas Tabari and Hadi Parsian designed and performed the experiments and also wrote the manuscript.data analysis by Mahmood Hajiahmadi. All authors read and approved the final version.

REFERENCES

Abdulle LE, Hao J-l, Pant OP, Liu X-f, Zhou D-d, Gao Y, et al. MALAT1 as a diagnostic and therapeutic target in diabetes-related complications: a promising long-noncoding RNA.International Journal of Medical Sciences. 2019; 16(4):548.

Al-Musharaf S, Sabico S, Hussain SD, Al-Tawashi F, AlWaily HB, Al-Daghri NM, et al. Inflammatory and Adipokine Status from Early to Midpregnancy in Arab Women and Its Associations with Gestational Diabetes Mellitus. Disease Markers. 2021; 2021.

Amirian A, Mahani MB, Abdi F. Role of interleukin-6 (IL-6) in predicting gestational diabetes mellitus.Obstetrics & Gynecology Science. 2020;63(4):407-16.

Baz B, Riverline J-P, Gautier J-F. Gestational diabetes mellitus: definition, etiological, and clinical aspects. Eur J Endocrinol. 2016;174(2): R43-51.

Biswas S, Thomas AA, Chen S, Aref-Eshghi E, Feng B, Gonder J, et al. MALAT1: an epigenetic regulator of inflammation in diabetic retinopathy. Scientific reports. 2018;8(1):1-15.

Braga FO, Negrato CA, MattaMdFBd, Carneiro JRI, Gomes MB.Relationship between inflammatory markers, glycated hemoglobin, and placental weight with fetal outcomes in women with gestational diabetes.Archives of endocrinology and metabolism. 2019;63(1):22-9.

Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics e-book: Elsevier Health Sciences; 2012.

Chang C-P, Han P. Epigenetic and lncRNA regulation of cardiac pathophysiology. BiochimicaetBiophysicaActa (BBA)-Molecular Cell Research. 2016;1863(7):1767-71.

Chen H, Wang X, Yan X, Cheng X, He X, Zheng W. LncRNA MALAT1 regulates sepsis-induced cardiac inflammation and dysfunction via interaction with miR-125b and p38 MAPK/NFκB. International immunopharmacology. 2018;55:69-76.

Chen X, Yang G-Y. Novel human lncRNA–disease association inference based on lncRNA expression profiles. Bioinformatics. 2013;29(20):2617-24.

Chiefari E, Arcidiacono B, Foti D, Brunetti A. Gestational diabetes mellitus: an updated overview. Journal of endocrinological investigation. 2017;40(9):899-909.

De Luca C, Olefsky JM.Inflammation and insulin resistance.FEBS letters. 2008;582(1):97-105.

Dennedy MC, Dunne F. The maternal and fetal impacts of obesity and gestational diabetes on pregnancy outcome. Best Practice & Research Clinical Endocrinology & Metabolism. 2010;24(4):573-89.

Desoye G, Hauguel-de Mouzon S. The human placenta in gestational diabetes mellitus: the insulin and cytokine network. Diabetes care. 2007;30(Supplement 2):S120-S6.

Dodington DW, Desai HR, Woo M. JAK/STAT–emerging players in metabolism.Trends in Endocrinology & Metabolism. 2018;29(1):55-65.

Ghayor C, Weber FE. Epigenetic regulation of bone remodeling and its impact in osteoporosis.International Journal of Molecular Sciences. 2016;17(9):1446.

Gomes CP, Torloni MR, Gueuvoghlanian-Silva BY, Alexandre SM, Mattar R, Daher S. Cytokine levels in gestational diabetes mellitus: a systematic review of the literature.American Journal of Reproductive Immunology. 2013;69(6):545-57.

Gordon AD, Biswas S, Feng B, Chakrabarti S. MALAT1: a regulator of inflammatory cytokines in diabetic complications.Endocrinology, diabetes & metabolism. 2018;1(2):e00010.

Goyal A, Gupta Y, Singla R, Kalra S, Tandon N. American Diabetes Association "Standards of Medical Care—2020 for Gestational Diabetes Mellitus": A Critical Appraisal. Diabetes Therapy. 2020;11(8):1639-44.

Gutschner T, Hämmerle M, Diederichs S. MALAT1—a paradigm for long noncoding RNA function in cancer. Journal of Molecular Medicine. 2013;91(7):791-801.

Gutschner T, Hämmerle M, Diederichs S. MALAT1—a paradigm for long noncoding RNA function in cancer. Journal of Molecular Medicine. 2013;91(7):791-801.

Kim C. Maternal outcomes and follow-up after gestational diabetes mellitus.Diabetic Medicine. 2014;31(3):292-301.

Kim JH, Bachmann RA, Chen J. Interleukin-6 and insulin resistance. Vitamins & Hormones. 2009;80:613-33.

Kuzmicki M, Telejko B, Szamatowicz J, Zonenberg A, Nikolajuk A, Kretowski A, et al. High resistin and interleukin-6 levels are associated with gestational diabetes mellitus.Gynecological endocrinology. 2009;25(4):258-63.

Lappas M, Mittion A, Permezel M. In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in the placenta, but not adipose tissue, of women with gestational diabetes.Journal of Endocrinology. 2010;204(1):75.

Lindsay KL, Buss C, Wadhwa PD, Entringer S. The effect of a maternal Mediterranean diet in pregnancy on insulin resistance is moderated by maternal negative effect. Nutrients. 2020;12(2):420.

Mack LR, Tomich PG. Gestational diabetes: diagnosis, classification, and clinical care. Obstetrics and Gynecology Clinics. 2017;44(2):207-17.

McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nature reviews Disease primers. 2019;5(1):1-19.

McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. Nature reviews Disease primers. 2019;5(1):1-19.

MORISSET AS, DUBÉ MC, Cote JA, Robitaille J, Weisnagel SJ, Tchernof A. Circulating interleukin-6 concentrations during and after gestational diabetes mellitus. ActaobstetriciaetgynecologicaScandinavica. 2011;90(5):524-30.

Mpondo BC, Ernest A, Dee. Gestational diabetes mellitus: challenges in diagnosis and management. Journal of Diabetes & Metabolic Disorders. 2015;14(1):1-7.

Nergiz S, Altınkaya ÖS, Küçük M, Yüksel H, Sezer SD, Kurt Ömürlü İ, et al. Circulating galanin and IL-6 concentrations in gestational diabetes mellitus. Gynecological Endocrinology. 2014;30(3):236-40.

Osgood ND, Dyck RF, Grassmann WK. The inter-and intragenerational impact of gestational diabetes on the epidemic of type 2 diabetes. American Journal of Public Health. 2011;101(1):173-9.

Özyer Ş, Engin-Üstün Y, Uzunlar Ö, Katar C, Danışman N. Inflammation and glycemic tolerance status in pregnancy: the role of maternal adiposity. Gynecologic and obstetric investigation. 2014;78(1):53-8.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT–PCR.Nucleic acids research. 2001;29(9):e45-e.

Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus.International Journal of Molecular Sciences. 2018;19(11):3342.

Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH.The pathophysiology of gestational diabetes mellitus.International Journal of Molecular Sciences. 2018;19(11):3342.

Pöyhönen-Alho M, Ebeling P, Saarinen A, Kaaja R. Decreased variation of inflammatory markers in gestational diabetes. Diabetes/metabolism research and reviews. 2011;27(3):269-76.

Ryan E. diagnosed gestational diabetes. Diabetologia. 2011;54(3):480-6.

Sun YY, Juan J, Xu QQ, Su RN, Hirst JE, Yang HX. Increasing insulin resistance predicts adverse pregnancy outcomes in women with gestational diabetes mellitus. Journal of Diabetes. 2020;12(6):438-46.

Tumurbaatar B, Poole AT, Olson G, Makhlouf M, Sallam HS, Thukuntla S, et al. Adipose tissue insulin resistance in gestational diabetes. Metabolic syndrome and related disorders. 2017;15(2):86-92.

Wang Y, Wang L, Guo H, Peng Y, Nie D, Mo J, et al. Knockdown of MALAT1 attenuates high-glucose-induced angiogenesis and inflammation via endoplasmic reticulum stress in human retinal vascular endothelial cells. Biomedicine & Pharmacotherapy. 2020;124:109699.

Yoshimoto R, Mayeda A, Yoshida M, Nakagawa S. MALAT1 is long noncoding RNA in cancer. BiochimicaetBiophysicaActa (BBA)-Gene Regulatory Mechanisms. 2016;1859(1):192-9.

Yu H, Liu Z, Dong S. Changes in intestinal flora, TNF-α, L-17, and IL-6 levels in patients with gestational diabetes mellitus. European Journal of Inflammation. 2018;16:2058739218793550.

Zhang J, Chi H, Xiao H, Tian X, Wang Y, Yun X, et al. Interleukin 6 (IL-6) and tumor necrosis factor α (TNF-α) single nucleotide polymorphisms (SNPs), inflammation and metabolism in gestational diabetes mellitus in Inner Mongolia. Medical Science Monitor: International medical journal of experimental and clinical research. 2017;23:4149.

Zhang Y, Qu L, Ni H, Wang Y, Li L, Yang X, et al. Expression and function of lncRNA MALAT1 in gestational diabetes mellitus. AdvClinExp Med. 2020;29:903-10.

Zhang Y, Wu H, Wang F, Ye M, Zhu H, Bu S. Long non-coding RNA MALAT 1 expression in patients with gestational diabetes mellitus. International Journal of Gynecology & Obstetrics. 2018;140(2):164-9.

Zhao X, Liu J, Shen L, Wang A, Wang R. Correlation between inflammatory markers (hs-CRP, TNF-α, IL-1β, IL-6, IL-18), glucose intolerance, and gestational diabetes mellitus in pregnant women. Int J ClinExp Med. 2018;11(8):8310-6.

Zhou L-j, Yang D-w, Ou L-N, Guo X-R, Wu B-l. Circulating Expression Level of LncRNA Malat1 in Diabetic Kidney Disease Patients and Its Clinical Significance.Journal of Diabetes Research. 2020;2020.

Zhu Y, Hedderson MM, Quesenberry CP, Feng J, Ferrara A. Central obesity increases the risk of gestational diabetes partially through increasing insulin resistance. Obesity. 2019;27(1):152-60.

Zhu Y, Zhang C. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective.Current diabetes reports. 2016.