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ORIGINAL RESEARCH

Long Non-Coding RNA HOTAIR Expression and Clinical Significance in Patients with Gestational Diabetes

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Purpose: The global incidence of gestational diabetes mellitus (GDM) is increasing year by year, and many studies have proved that long non-coding RNA (lncRNA) is involved in the regulation of GDM. The purpose of this study was to investigate the expression of HOTAIR in GDM patients and its clinical significance.

Patients and Methods: Ninety-eight healthy pregnant women and 99 pregnant women diagnosed with GDM were enrolled in this study. Blood samples were collected from all participants and used for qRT-PCR analysis to determine the serum HOTAIR levels. The ROC curve was constructed to evaluate the diagnostic value of HOTAIR for GDM. Pearson correlation coefficient was used to estimate the correlation between HOTAIR and clinical indicators of patients. Logistic regression analysis was performed to evaluate the independent predictors of GDM.

Results: The level of HOTAIR was augmented in GDM group compared with healthy controls. ROC curve revealed that HOTAIR as a diagnostic marker of GDM has high sensitivity and specificity. Pearson correlation coefficient showed that HOTAIR level was positively correlated with body mass index, fasting plasma glucose, 1-hour plasma glucose and 2-hour plasma glucose. Logistic regression analysis shows that HOTAIR is an independent factor of the occurrence of GDM.

Conclusion: The abnormal expression of HOTAIR in pregnant women with GDM made it a potential diagnostic biomarker for GDM.

Keywords: gestational diabetes mellitus, HOTAIR, diagnosis, biomarker

Introduction

Gestational diabetes mellitus (GDM) is an independent type of diabetes mellitus, which refers to the abnormal glucose tolerance of pregnant women or is first discovered during pregnancy, and it is one of the most common complications in the first and second stages of pregnancy.[1,2] In China, the incidence of diabetes is rapidly rising, while the incidence of GDM is about 12.2%.[3] During GDM, pregnant women will have abnormal blood glucose regulation, lipid metabolism, immune function and cardiopulmonary function to varying degrees,[4] and may have pregnancy-related complications, such as pregnancy-related hypertension, eclampsia or abortion, premature birth, malformation.[5] Although blood glucose level in patients with GDM usually returns to normal after delivery, in the long term, women with a history of GDM are more likely to develop type 2 diabetes,[6] and their babies are more likely to be overweight or obese later in life.[7] Therefore,

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prevention and early diagnosis of GDM are of great important to avoid adverse effects. In the past, scholars tried to identify and search for biomarkers of GDM, such as HbA1c[8] and adiponectin.[9] However, they were not effectively applied in clinical practice due to various reasons.

Long non-coding RNAs (lncRNAs) have attracted the attention of scholars under the current research background. LncRNAs are a class of functional RNA molecules with a length of more than 200 nucleotides, which regulate gene expression at the epigenetic, transcriptional and post-transcriptional levels and extensively participate in most processes of the organism growth.[10,11] Numerous studies have proved that lncRNAs are involved in the occurrence and development of diseases, including cancer,[12] cardiovascular disease,[13] and autoimmune disease.[14] LncRNA HOTAIR is located on chromosome 12q13, and it has been found to be abnormally expressed in various cancers.[15] Existing studies have demonstrated that HOTAIR is elevated in the liver tissues of patients with type 2 diabetes mellitus (T2DM) and mice fed with a high-fat diet. In addition, the upregulation of HOTAIR promotes hepatic insulin resistance through the AKT/GSK pathway.[16] Another study confirmed that levels of HOTAIR in PBMCs were significantly enhanced in patients with T2DM compared with normal glucose tolerance group.[17] At present, the evidence has confirmed the association between HOTAIR and T2DM, but there are few studies on the correlation between HOTAIR and GDM.

In our study, blood samples were collected to measure the expression of HOTAIR in GDM and healthy pregnant women. In addition, we also studied the clinical diagnostic value of HOTAIR in GDM and evaluated the correlation between HOTAIR and clinical indicators as well as the occurrence of GDM.

Materials and Methods

Patients and Samples

A total of 197 pregnant women were enrolled in this study, among whom 99 were GDM patients, who were admitted to The Central Hospital of Enshi Tujia and Miao Autonomous Prefecture between February 2020 and March 2021. The diagnosis of GDM is based on the GDM diagnostic guidelines formulated by the American Diabetes Association.[18] Inclusion criteria: fasting blood-glucose ≥5.1 mmol/L. And/or oral glucose tolerance test (OGTT) with 1-hour blood-glucose ≥10.0 mmol/L, and/or 2-hour blood-glucose ≥8.5 mmol/L. Another 98 pregnant women were healthy and were defined as healthy controls. Exclusion criteria: Subjects with pregestational diabetes, abnormal liver function, severe infection, and malignant tumors were excluded from the study. All subjects underwent the same physical examination and OGTT examination. General information and clinicopathological indicators of the participants were recorded. Venous blood of each subject was collected, centrifuged and stored in the refrigerator at −80°C for further use. This study was conducted in accordance with the Declaration of Helsinki, and it has been approved by the Ethics Committee of The Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, and all subjects have signed written informed consent.

qRT-PCR Analysis

Total RNAs in serum were extracted by TRIzol reagent (Invitrogen), and the reverse transcription of RNA was performed by SuperScript II Reverse Transcriptase kit (Invitrogen, USA) according to the manufacturer's protocols. The amplification of cDNA was performed on the Applied Biosystems 7900 Real-Time PCR System using miScript SYBR® Green PCR kit (Qiagen GmbH, Germany). Amplification primers for qRT-PCR analysis are as follows: HOTAIR: (forward primer): 5′-TCGCAGTGGAATGGAACGGA-3′, (reverse primer): 5′-GCGACCGGAGCTCATCTTAC-3′. GAPDH: (forward primer): 5′-CGCTGAGTACGTCGTGGAGT-3′, (reverse primer): 5′-TGTCATCATATCTGGCAGGT-3′. Using GAPDH as internal reference, the relative expression level of HOTAIR was calculated by $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The differences between groups were analyzed by Student *t*-test or one-way ANOVA. ROC curve was used to evaluate the clinical diagnostic value of HOTAIR in GDM. Pearson correlation coefficient analyzed the correlation between HOTAIR expression and clinical indicators in GDM patients. Logistic regression was used to assess the relationship between different variables and the occurrence of GDM. Statistical analysis of this study was conducted using SPSS 17.0 software and GraphPad Prism 7 software. Data conforming to the normal distribution were expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Comparison of Baseline Data

The baseline data and clinical indicators of participants are shown in Table 1. We could see that there were significant differences in body mass index (BMI), fasting plasma glucose (FBG), 1-hour plasma glucose, 2-hour plasma glucose, total cholesterol, and triglyceride between healthy pregnant women and pregnant women with GDM ($P < 0.001$). There were no significant differences in age, pregnancy, fetal birth weight and cholesterol between the two groups.

Serum Level of HOTAIR in Pregnant Women with GDM

In this study, qRT-PCR was used to compare the expression levels of HOTAIR in serum of healthy pregnant women and pregnant women with GDM. The expression level of serum HOTAIR in pregnant women with GDM was augmented evidently in comparison to those healthy pregnant women (Figure 1, $P < 0.001$). We speculated that the abnormal expression of HOTAIR may play a role in GDM occurrence.

ROC Analysis

ROC curve was used to analyze the diagnostic value of HOTAIR in GDM. Results indicated that the AUC value was 0.906 (Figure 2, sensitivity = 83.8%, specificity = 83.7%), suggesting that HOTAIR level had the ability to distinguish pregnant women with GDM and healthy pregnant women.

Pearson Correlation Coefficient and Logistic Regression Analysis

Pearson correlation coefficient was performed to analyze the correlation between HOTAIR expression and clinicopathological indexes in pregnant women with GDM. As shown in Table 2, HOTAIR levels were significantly positively correlated with BMI, FBG, 1-hour plasma glucose and 2-hour plasma glucose levels ($P < 0.01$), which suggested that abnormal expression of HOTAIR was related to GDM and was also associated with the severity of GDM. In addition, logistics regression analysis was used to evaluate the relationship between different variables and the GDM occurrence. We found that HOTAIR (OR = 20.939, 95% CI = 9.415–46.567, $P < 0.001$) and BMI (OR = 2.999, 95% CI = 1.348–6.670, $P = 0.007$) were the independent influencing factors of GDM occurrence among many variables (Table 3).

Discussion

Our study suggested that the expression of HOTAIR in pregnant women with GDM was significantly enhanced compared with that of healthy pregnant women, and that the abnormal expression of HOTAIR had higher clinical diagnostic value for GDM. Moreover, it was also found that HOTAIR level was positively correlated with the level of FBG and other indicators in pregnant women with GDM, which was an independent factor of the occurrence of GDM.

GDM is a disease that can cause serious complications for both a mother and her child.[19] Timely prevention and treatment of GDM may help limit the long-term burden of

Table 1 Clinical Data of the Subject Population

Characteristics	Healthy Pregnant Women (n=98)	Gestational Diabetes Mellitus (n=99)	*P*-value
Age (years)	29.12±1.10	29.39±1.05	0.77
BMI (kg/m^2)	25.00±3.07	27.82±4.51	<0.001
Gestation (week)	26.79±1.18	26.72±1.65	0.747
Fasting plasma glucose (mM)	4.52±0.32	5.30±0.11	<0.001
One-hour plasma glucose (mM)	6.27±0.21	10.21±0.27	<0.001
Two-hour plasma glucose (mM)	5.47±0.42	8.28±0.19	<0.001
Fetal birth weight (g)	3409.35±263.52	3430.46±202.66	0.529
Total cholesterol (mM)	5.49±0.41	5.91±0.46	<0.001
Triacylglycerol (mM)	0.99±0.33	1.28±0.34	<0.001
Low-density lipoprotein cholesterol (mM)	2.62±0.43	2.73±0.41	0.109

Note: Data are expressed as n or mean ± standard deviation.
Abbreviation: BMI, body mass index.

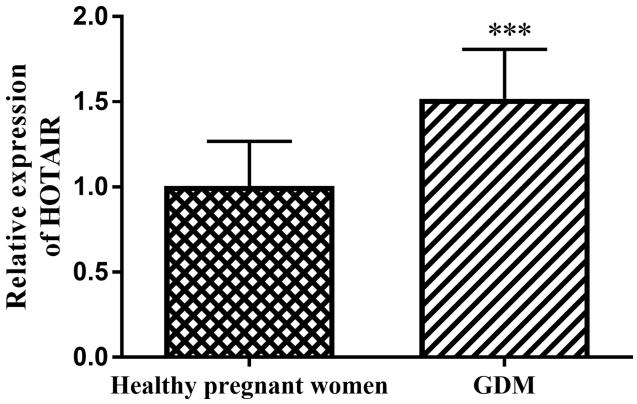

Figure 1 HOX transcript antisenseRNA (HOTAIR) expression was enhanced in pregnant women with gestational diabetes mellitus (GDM) (***$P < 0.001$).

the disease.[20] Therefore, researchers are increasingly interested in finding new biomarkers with diagnostic, prognostic, and therapeutic effects in GDM. LncRNA plays a particularly important regulatory role in the growth, development, and metabolism of the organism. It has been well established that lncRNAs are involved in glucose metabolism, fat formation, and endocrine gland function.[21] For example, lncRNA GAS5 can inhibit 6-phosphogluconase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), which are the key enzymes in gluconeogenesis.[22] Xu et al found that lncRNA SRA regulates adipogenesis through activation of insulin/IGF-1 signaling pathway.[23] In our study, HOTAIR was upregulated in serum of pregnant women with GDM, and its expression level was positively correlated with BMI, plasma glucose and lipid levels of the patients. It was reported that HOTAIR was first discovered in human fibroblast HOX gene. Recent studies have shown that HOTAIR was highly expressed in adipose tissue, and in clinical sample analysis, circulating exosomes HOTAIR was higher in the obese group than in the lean group.[24] The distribution of body fat has extensive repeatability with metabolic health, and obesity is closely related to the development of cardiovascular diseases and diabetes.[25,26] Under these circumstances, we preliminarily speculated that the production of high levels of HOTAIR in GDM pregnant women may be indirectly attributable to unfriendly indicators such as BMI. Therefore, we conducted that the level of HOTAIR was closely related to the degree of obesity and the severity of GDM.

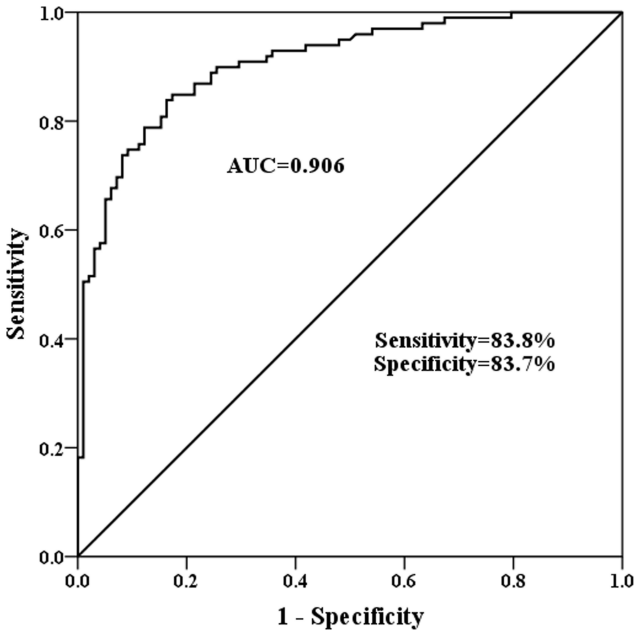

Figure 2 Receiver operating characteristic (ROC) curve was drawn to estimate the diagnostic significance of HOX transcript antisenseRNA (HOTAIR) in gestational diabetes mellitus (GDM).

ROC curve indicated that HOTAIR levels in blood samples could be used as a factor to distinguish GDM pregnant women from healthy pregnant women. Unlike most protein biomarkers, lncRNAs are stable in blood circulation,[27] and many previous studies have pointed out the important role of lncRNAs in the early diagnosis of diseases.[28,29] Accordingly, our study preliminarily identified the potential of HOTAIR as a diagnostic biomarker for GDM diagnosis. Furthermore, logistic regression analysis exhibited that HOTAIR and BMI were independent factors of GDM, respectively. As far as BMI is concerned, the association between diabetes and obesity or overweight is self-evident.[30] Obesity or overweight has been considered as an important risk factor for T2DM. T2DM will not only increase insulin resistance,[31] but also aggravate metabolic abnormalities, including hyperglycemia, hyperlipidemia, and hyperinsulinemia.[32] For HOTAIR, Shaker et al demonstrated that HOTAIR level was significantly enhanced in serum of diabetic retinopathy patients compared with non-diabetic retinopathy patients.[33] According to reports, HOTAIR acts as a sponge molecule of miR-34 in diabetic cardiomyopathy, and aggravates the condition by promoting the expression of SIRT1.[34] Malumder et al found that HOTAIR was expressed in both human and mouse renal tissues and increased in the kidneys of streptozotocin-induced diabetes mice and diabetic nephropathy patients.[35] In the previously published studies listed above, the expression of HOTAIR was enhanced dramatically in both T2DM and diabetic-related diseases, and HOTAIR level in serum of pregnant women with GDM in this study was consistent with these results.

Table 2 Correlation Between lncRNA HOTAIR and Clinical Characteristics

Characteristics	Correlation with lncRNA HOTAIR	*P*-value
Age (years)	0.152	0.132
BMI (kg/m^2)	0.297	0.003
Gestation (week)	0.068	0.506
Fasting plasma glucose (mM)	0.614	<0.001
One-hour plasma glucose (mM)	0.588	<0.001
Two-hour plasma glucose (mM)	0.417	<0.001
Fetal birth weight (g)	0.023	0.819
Total cholesterol (mM)	0.070	0.187
Triacylglycerol (mM)	0.134	0.491
Low-density lipoprotein cholesterol (mM)	0.095	0.352

Abbreviation: BMI, body mass index.

Table 3 Association of Different Variables with the Occurrence of Gestational Diabetes Mellitus

Characteristics	OR	95% CI	*P* value
LncRNA HOTAIR	20.939	9.415–46.567	<0.001
Age (years)	1.422	0.611–3.310	0.414
BMI (kg/m^2)	2.999	1.348–6.670	0.007
Gestation (week)	1.954	0.853–4.476	0.113
Fetal birth weight (g)	1.380	0.629–3.028	0.421
Total cholesterol (mM)	1.777	0.770–4.103	0.178
Triacylglycerol (mM)	2.009	0.897–4.497	0.090
Low-density lipoprotein cholesterol (mM)	1.073	0.474–2.429	0.886

Abbreviation: BMI, body mass index.

LncRNA can be used as miRNA sponge to regulate the disease progression by regulating the expression of target mRNA downstream of miRNA. For example, HOTAIR participated in promoting the progression of advanced gastric cancer by inhibiting the expression of HER2 through sponge miR-331-3p.[36] At present, there are still relatively few studies on the mechanism of HOTAIR in GDM, and this study has not further explored the downstream target genes of HOTAIR based on the current data. However, in terms of the current experimental data, this study only made a preliminary analysis and explanation based on the expression of HOTAIR in the serum of pregnant women with GDM and healthy pregnant women. In order to improve the scientific and guiding significance of this study, we also need to determine whether the expression of HOTAIR in pregnant women's placenta or amniotic fluid is consistent with that in blood, as well as the mechanism of HOTAIR in GDM, and verify our further results through in vitro or in vivo experiments. For these issues, we should put forward and develop solutions in the future related research.

Conclusion

In summary, the results of this study suggest that HOTAIR is upregulated in pregnant women with GDM, and the evaluation results of ROC curve indicate that HOTAIR is of high diagnostic value for GDM. In addition, HOTAIR proved to be an independent factor in the occurrence of GDM. Therefore, this study may provide evidence for finding new diagnostic markers in clinical application.

Ethics Statement

This study has been approved by the Ethics Committee of The Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, and all subjects have signed written informed consent.

Disclosure

The authors report no conflicts of interest in this work.

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