Sitagliptin's Effect on MALAT1 in T2DM Patients Experiencing Diastolic Failure of the Left Ventricle

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ABSTRACT

Background: Patients with type 2 diabetes may develop cardiovascular disease (CVD) without experiencing or recognizing the characteristic signs and symptoms. Diastolic dysfunction is present in 40% of individuals diagnosed with diabetes and is associated with inadequate glycemic management. The MALAT1 coding gene is situated on the short arm of human chromosome 11q13.1, and its transcript spans around 8 kilo bites. MALAT1 has substantial involvement in the pathophysiological process. It performs the roles of an innovative biomarker and target of therapy for the diagnosis, treatment, and prognosis of diabetes-related conditions. The expression of MALAT1 is much higher in the heart tissue of rats with diabetes mellitus (DM), and inhibiting MALAT1 improves cardiac function.

Objective: To clarify the role of sitagliptin in left ventricular diastolic dysfunction in T2DM and investigate its potential impact on epigenetic modulation (lnRNA MALAT1) and the inflammatory process.

Materials and Methods: This study is an observational cross-sectional descriptive study conducted at a single center. Sixty individuals with type 2 diabetes mellitus and left ventricular diastolic dysfunction in total were recruited from AL-Diwaniyah Teaching Hospital and the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Al-Qadisiyah, Iraq, under the supervision of a specialist cardiologist. The Trizol reagent (Genaid, Korea) was used to extract the total RNA, and the concentration of RNA was subsequently determined. The primer amplification was carried out in accordance with the manufacturer's instructions for the AddScript RT-qPCR Syber master (AddBio, Korea).

Results: study results indicate a significant downregulation of MALAT1 in the sitagliptin-treated group ($P<0.0001$) compared to metformin treatment. This was analyzed by GraphPad Prizm software (version 8.4.3).

Conclusion and recommendation: The present study's findings indicate that the expression of MALAT1 is notably increased in the cardiac tissue of individuals with diabetes. Additionally, reducing the expression of MALAT1 is linked to an enhancement in the functioning of the left ventricle, partially due to a decrease in programmed cell death of heart muscle cells. Hence, suppressing MALAT1 could be a promising therapeutic approach for managing cardiac dysfunction associated with diabetes.

Keywords: Type 2 Diabetic mellitus, Left ventricular diastolic dysfunction , Long non-coding RNA, MALAT1, Sitagliptin

تأثير سيتاجليبتن على MALAT1 في مرضى السكري النوع الثاني الذين يعانون من عجز عضلة البطين الأيسر الانبساطي

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الخلاصة

الخلفية: قد يصاب مرضى السكري من النوع الثاني بأمراض القلب والاوعية الدموية دون تمييز او ظهور الاعراض المهمة. اعتلال عضلة القلب الانبساطي لوحظ لدى٤٠ % من مرضى السكري المرتبط مع ضعف السيطرة على نسبة السكر في الدم. يقع الجين المشفر MALAT1 على الذراع القصيرة للكروموسوم البشري11q13.1 ، ويستنسخ الى حوالي ٨ كيلو بايت. يمكن أن يلعب MALAT1 أدوارا مهمة في العملية الفسيولوجية المرضية، حيث يعمل MALAT1 كمؤشر حيوي مبتكر وهدف علاجي لتشخيص وعلاج المضاعفات المرتبطة بمرض السكري وتقييم تشخيص المرض. يظهر MALAT1 بشكل مفرط إلى حد كبير في الأنسجة القلبية للفئران المصابة بمرض السكري، ويؤدي تثبيطه إلى تحسن في وظيفة القلب.

الهدف: لتوضيح دور عقار سيتاجليبتين على الاعتلال الانبساطي لعضلة البطين الأيسر لدى مرضى السكري النوع الثاني، والتحري عن الدور المحتمل للسيتاجليبتين في التعديل اللاجيني (lnRNA MALAT1) والعملية الالتهابية.
المواد والطرق: هذه دراسة وصفية مقطعية رصدية أحادية المركز ل٦٠ مريضا يعانون من مرض السكري النوع الثاني مع اعتلال عضلة القلب الانبساطي للبطين الايسر تم تشخيصهم من قبل اخصائي امراض القلب في مستشفى الديوانية التعليمي وفرع الادوية والعلاجيات في كلية الطب جامعة القادسية في العراق. تم استخراج اجمالي الحمض النووي وقياس تركيزه باستخدام كاشف تريزول (جينيد، كوريا)، وتم قياس تركيز الحمض النووي الريبي. تم إجراء تضخيم التمهيدي وفقا لبروتوكولات الشركة المصنعة AddScript RT-qPCR Syber master (AddBio ، كوريا).
النتائج: تشير نتائج الدراسة الى حدوث انخفاض كبير في تركيز MALAT1 في المجموعة المعالجة بالسيتاجليبتين (P<0.0001) مقارنة بعلاج الميتفورمين. تم تحليل هذا بواسطة برنامج Graph pad prizim (الإصدار ٨.٤.٣).
الاستنتاج: تشير استنتاجات الدراسة الى انMALAT1 مرتفع بشكل كبير في الأنسجة القلبية لمرضى السكري، ويرتبط تثبيطه بتحسن في وظيفة البطين الأيسر، جزئيا من خلال موت الخلايا المبرمجة لعضلة القلب. لذلك، قد يكون تثبيط MALAT1 بمثابة استراتيجية علاجية جديدة لضعف القلب المرتبط بمرض السكري.

الكلمات المفتاحية : مرض السكري النوع الثاني، اعتلال عضلة البطين الايسر، الحمض النووي الطويل غير المشفر، MALAT1, سيتاجليبتين.

INTRODUCTION

Type 2 diabetes mellitus (T2DM), previously referred to as non-insulin dependent diabetes mellitus or adult-onset diabetes, is a metabolic condition characterized by high blood sugar levels and reduced insulin sensitivity. These variables lead to cardiovascular risk factors, including abnormal lipid levels, high blood pressure, and obesity [1].

Arises from the interplay of lifestyle and genetic influences. According to the International Diabetes Federation (IDF), approximately 1 in 11 persons are affected by DM, with 90% being T2DM. Asia is considered the epicenter of this global epidemic of T2DM [2]. The Middle East has a significant occurrence of T2DM in men, with Bahrain having a prevalence of about 33%, Saudi Arabia at approximately 29%, the United Arab Emirates at 25%, and Iraq at 13% [3,4].

Individuals with type 2 diabetes may develop cardiovascular disease (CVD) without exhibiting or acknowledging the characteristic indications and symptoms.

Recent investigations have shown that diastolic dysfunction occurs in 30-75% of diabetic patients [5].

Left ventricular diastolic dysfunction (LVDD) is a preclinical state characterized by the left ventricle's failure to adequately fill with blood during relaxation (diastole) at a suitable pressure. To delay or prevent the onset of cardiac failure in individuals with type 2 diabetes, it is crucial to identify risk factors for LV diastolic dysfunction and an index of early-stage diabetic cardiomyopathy, considering the high prevalence and severe morbidity and mortality associated with heart failure in these patients [6].

Metabolic syndrome (MetS), as well as systemic disorders such as chronic obstructive pulmonary disease (COPD), atrial fibrillation (AF), and anemia, are considered classical cardiovascular risk factors due to their inflammatory nature [7].

Diastolic dysfunction is present in 40% of individuals diagnosed with diabetes and is associated with inadequate glycemic management.

Left ventricular diastolic dysfunction (LVDD) is widely regarded by scientists as the initial indication of cardiac remodeling in individuals with DM. LVDD primarily involves impaired relaxation and increased stiffness of the left ventricle. These abnormalities are likely caused by alterations in the quantity and/or quality of calcium regulatory proteins and the extracellular matrix [8]. Long non-coding RNAs (LncRNAs) that play a role in maintaining glucose balance contribute to the advancement of diabetes and related disorders. Studies have demonstrated that Asians have a higher level of insulin resistance and are more likely to develop T2DM and associated vascular complications compared to those of other races [9]. The MALAT1 coding gene is situated on the short arm of human chromosome 11q13.1, and its transcript spans around 8 kilo bites. MALAT1 has been associated with a range of pathological processes, such as diabetes-related problems and different types of malignancies [10].

MALAT1 significantly influences various pathophysiological processes, including tissue inflammation, tumor progression, angiogenesis, cardiovascular remodeling, liver fibrosis, and diabetes progression, through its modulation of gene transcription. MALAT1 functions as a novel biomarker and therapeutic target for the diagnosis and treatment of problems associated with diabetes, as well as for assessing the prognosis of the disease [11]. MALAT1 is crucial in enhancing the left ventricular function in diabetic rats.

The involvement of high glucose-induced cardiomyocyte apoptosis in the development of diabetic cardiomyopathy has been well described [12]. Reducing the expression of MALAT1 might significantly reduce the concentration of inflammatory cytokines, suggesting that MALAT1 may have a role in the development of DCM.

Suppressing MALAT1 could be a promising therapeutic approach for treating cardiac dysfunction associated with diabetes.

The abundant presence of DPP4 in the blood vessels, heart muscle, and immunological cells indicates that this protein might be involved in cardiovascular function [13]. Sitagliptin is a medication that selectively inhibits the function of the dipeptidyl peptidase-4 (DPP-4) enzyme. Sitagliptin lowers the amount of glucose the liver produces and enhances the pancreas' sensitivity to glucose by inhibiting DPP-4 [14]. In diabetic rats, sitagliptin improves lipid content, collagen metabolism, inflammatory activity, myocardial apoptosis (RIP3 expression), and cardiac function. This aids in identifying the benefits of sitagliptin that go beyond its capacity to lower blood sugar levels.

DPP-IV, also known as CD26, is present on the surfaces of many cells, particularly leukocytes, where it acts as an inflammatory mediator. If DPP-IV and CD26 are proinflammatory, their inhibitors, such as sitagliptin, have the potential to be anti-inflammatory and may also have the capacity to reduce the risk of atherosclerosis, which is a chronic inflammation of the artery wall [15].

MATERIALS AND METHODS

Study Subjects

Sixty adults (25 male and 36 female) aged 20-75 years old were enrolled in this study. Patients with high HbA1c, renal or hepatic impairment, ejection fraction less than 50%, pregnancy, heart failure, obesity (BMI ≥ 30), and psychiatric patients were considered as exclusion criteria.

Ethical Approval

The Ethics Committee of the Medicine College, University of Al-Qadisiyah, approved the study procedures, which were explained to all participants, and consent was obtained from each candidate patient.

Drug used in the study: All patients took oral sitagliptin 50 mg and 1000 mg Metformin daily.

RNA was extracted from blood samples collected from the antecubital vein:

Using an RNA extraction kit from Genaid, Korea. The concentration of the extracted RNA was measured using a Quantus™ Fluorometer from Promega, USA. Subsequently, cDNA synthesis was performed using a cDNA synthesis kit from ADDBio, Korea.

Gene expression of MALAT1: This study utilized the comparative Ct technique (ΔΔCt) to compare transcript levels of the target gene to the control group while normalizing to the levels of GAPDH mRNA. This was accomplished in accordance with the suggestion provided by [16].

For this purpose, the MALAT-1 gene was amplified using the following primers employed from [17].

Gene of Interest (MALAT-1) is
MALAT1-Forward:
GGATCCTAGACCAGCATGCC and
MALAT1-Reverse:
AAAGGTTACCATAAGTAAGTTCCAGAAAA

Housekeeping gene (HKG) or internal reference gene; human Glyceraldehyde 3-phosphate dehydrogenase is GAPDH-F:
CAGAACATCATCCCTGCCTCTA
GAPDH-R: CCAGTGAGCTTCCCGTTCA
Quantitative Reverse transcriptase PCR (RT-qPCR) Preparation
RT-qPCR amplification:
A/ Initially, the amplification was accomplished using the AddScript RT-qPCR Syber master (AddBio, Korea).
B/ The thermal conditions were conducted using BioRAD (USA).
RT-qPCR data normalisation:

The transcript levels were normalized to the GAPDH mRNA using the delta-delta Ct method, as described by [16]. This method involved the application of the following formula:

2-ΔΔCT = [(CT gene of interest- CT internal control) sample A- (CT gene of interest- CT internal control) sample B)].

"Sample A" refers to a specific group. Sample B refers to a distinct and specific group.

Statistical Analysis

Data analysis was performed using GraphPad Prism software version 8.4.3. The data is shown as the average ± standard deviation (SD). A statistical significance was attributed to differences with a P value <0.05.

RESULTS

The efficiency of the assay's amplification curve in tested groups for (MALAT1).

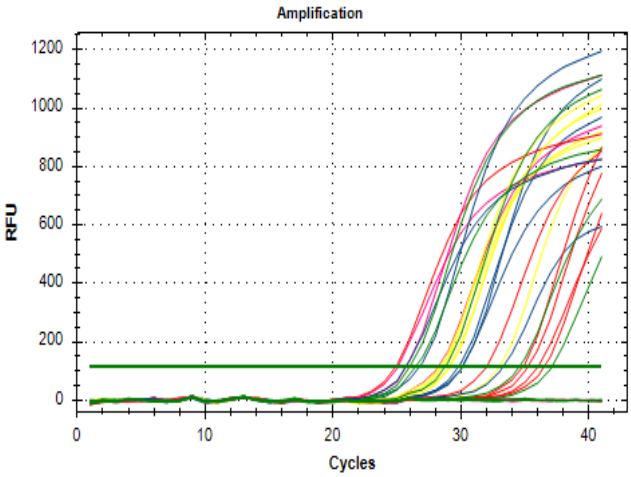

Figure (1): The amplification graphs demonstrate successful amplification, with associated crossing threshold values, as the number of cycles increases using the round-forming unit

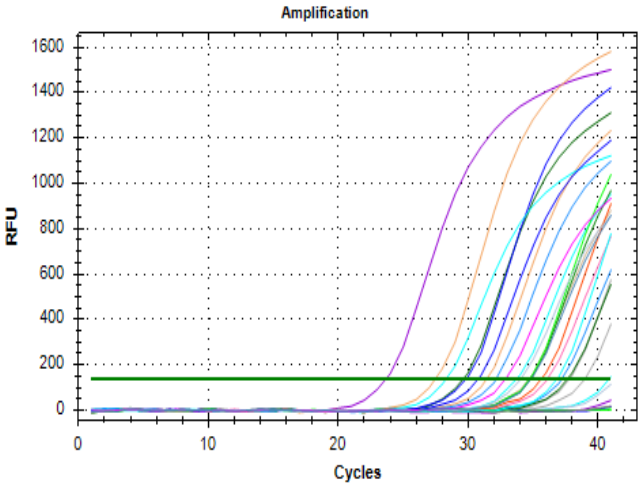

Figure (2) The amplification graphs demonstrate successful amplification, with associated crossing threshold values, as the number of cycles increases using the round forming unit.

Gene expression of MALAT1 in the sitagliptin group in comparison with Metformin:

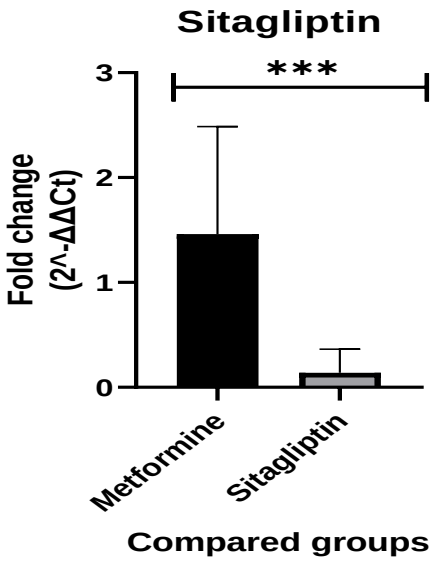

Figure (3): bar chart illustrating gene expression of MALAT1 in sitagliptin treated group in comparison with metformin treatment.

This shows a significant downregulation of MALAT1 in the sitagliptin-treated group (P<0.0001). The analysis was conducted using GraphPad Prism software (version 8.4.3).

Discussion: The current study aimed to examine the impact of MALAT1 on the left ventricular function in individuals with diabetes. The results of our study showed a correlation between MALAT1 and diabetic patients with left ventricular diastolic dysfunction who were treated with sitagliptin. We observed a significant decrease (p<0.0001) in MALAT1 concentration and improvement in echocardiographic parameters associated with LVDD in the sitagliptin group compared to the control group (Metformin alone). This suggests that sitagliptin may benefit as an additional therapy to Metformin. In another investigation, it was found that MALAT1 expression was significantly increased in diabetic rats [18]. Reducing the expression of MALAT1 might significantly lower the levels of inflammatory cytokines, suggesting that MALAT1 may play a role in the progression of DCM. In addition, the suppression of MALAT1 resulted in a substantial reduction in cardiomyocyte apoptosis in the group treated with MALAT1-shRNA in DM-positive individuals [19].

The expression of MALAT1 is significantly higher in the heart tissue of rats with diabetes mellitus (DM), and inhibiting MALAT1 leads to an enhancement in cardiac function. Therefore, inhibiting the expression of MALAT1 could be a promising and innovative treatment strategy for DC. In contrast, some investigations have shown that Malat-1 has no role in transcriptional alterations or total cardiac hypertrophy. Additionally, these studies have demonstrated that Malat-1 does not regulate myocardial capillary density in both normal physiological and pressure-overloaded settings.

CONCLUSIONS

1. The results of the present study indicate that the expression of MALAT1 is dramatically increased in the cardiac tissue of individuals with diabetes.
2. Reducing the expression of MALAT1 is linked to an improvement in the function of the left ventricle, partially due to a decrease in the death of heart muscle cells. Thus, suppressing MALAT1 could be a promising therapeutic approach for treating cardiac dysfunction associated with diabetes.
3. The expression of MALAT1 was considerably decreased when sitagliptin was added to Metformin as a treatment, compared to the control group receiving only Metformin.

RECOMMENDATIONS

additional research with bigger sample numbers and family-based analyses are needed to corroborate this association.

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