Empagliflozin's Effect on Micro RNA150-5p Expression in Individuals with Diastolic Dysfunction of the Left Ventricle in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Type 2 Diabetes Mellitus (T2DM) is one of the most prevalent metabolic diseases characterized by persistent hyperglycemia, and heart failure can be a common comorbidity and fatal complication of diabetes mellitus. An early sign of diabetic heart disease is left ventricular diastolic dysfunction, and microRNA dysregulation was one of the pathogenic mechanisms behind left ventricular diastolic dysfunction.

Aims: To investigate the potential effect of Empagliflozin on genetic modulation (Micro RNA150-5P).

Method: This is a single-center, cross-sectional, descriptive observational research. Specialist cardiologists recruited sixty individual T2D and left ventricular diastolic dysfunctions at AL-Diwaniyah Teaching Hospital, and the amount of RNA was quantified. The total RNA was extracted using Trizol reagent (Genaid, Korea), and the manufacturer's instructions to Add the RT-qPCR Syber master script were followed when performing the primer amplification. (Add Bio, Korea)

Result: Empagliflozin induces a considerable dawn regulation of miR-150-5p in left ventricular diastolic dysfunction, indicating that empagliflozin negatively affects microRNA 150-5p in diabetic patients with LVDD.

Conclusion: Empagliflozin causes a decrease in the expression of micro RNA in diabetic patients with left ventricular diastolic dysfunction, and this effect is statistically significant compared to metformin treatment.

Keywords: Type2diabetes mellitus ,Left ventricular diastolic dysfunction, Micro RNA .

تأثير علاج الامباجليفلوزين على تعبير (Micro RNA150-5P) لدى مرضى داء السكر النوع الثاني الذين لديهم خلل انبساطي للبطين الايسر

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الخلاصة

الخلفية : داء السكري من النوع ٢ (T2DM) هو اضطراب أيضي شائع يتميز بفرط سكر الدم المزمن ويمكن اعتبار فشل القلب هو اعتلال مشترك ومضاعفات مميتة لمرض السكري ويكون الخلل الوظيفي الانبساطي للبطين الأيسر (LV) هو مظهر مبكر لمرض القلب السكري وأحد الأعراض المبكرة لمرض السكري. وأحدى الآليات المرضية للخلل الوظيفي الانبساطي هي خلل تنظيم microRNA.

الأهداف : توضيح تأثير علاج الامباجليفلوزين على الخلل الوظيفي الانبساطي للبطين الأيسر في مرضى داء السكري من النوع الثاني، وكذلك دراسة التأثير المحتمل للامباجليفلوزين على التعديل الجيني لل (Micro RNA150-5P).

المواد والطرق : هذه دراسة وصفية مقطعية رصدية احاديه المركز ل٦٠ مريضا يعانون من داء السكري من النوع الثاني مع خلل وظيفي انبساطي للبطين الايسر وتم تشخيصهم من قبل الطبيب المختص في مستشفى الديوانية التعليمي في العراق . تم استخلاص RNA باستخدام ماده الترايزول (Genaid, Korea)وتم حساب تركيز RNA. وتم إجراء التضخيم التمهيدي وفقًا لبروتوكولات الشركة المصنعة(AddScript RT-qPCR Syber master (AddBio, Korea).

النتائج : بالمقارنة مع الميتفورمين، يتسبب إمباغليفلوزين في انخفاض كبير في تنظيم miR-150-5p في قصور القلب السكري، وهذا يعني أن إمباغليفلوزين له تأثيرسلبي على micro RNA 150-5p في مريض السكري.

الاستنتاج : كشفت هذه الدراسه إلى أن انخفاض تنظيم micro RNA 150-5P في قصور القلب السكري وعلاج الإمباجليفلوزين له تأثير سلبي على micro RNA 150-5p بالمقارنة مع علاج الميتفورمين .

الكلمات المفتاحية : داء السكري النوع الثاني، خلل وظيفي انبساطي للبطين الايسر، microRNA.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is prevalent and characterized by prolonged hyperglycemia. Hyperglycemia is brought on by impaired insulin synthesis, secretion, and action. Because of the increased risk of peripheral neuropathy, heart disease, stroke, kidney disease, blindness, and amputation, it is linked to a shortened life expectancy[1]. T2DM is a complicated disease impacted by genetic and environmental variables. The pathophysiological changes, characterized by β-cell dysfunction, insulin resistance, and chronic inflammation, lead to micro- and macrovascular complications. Blood glucose regulation is progressively hampered by these factors[2]. Based on data released between 1990 and 2018, the estimated incidence of diabetes mellitus (DM) in the Iraqi people was approximately 9 %. By 2030 and 2045, an increase to 10 % and 10.9% is anticipated[3]. T2DM and hyperglycemia have a significant impact on the development and prognosis of diastolic heart failure. Left ventricular diastolic dysfunction (LVDD) is believed to be an early sign of diabetic heart disease[4]. According to a prior study, diastolic dysfunction has been connected to an HbA1c > 8, but treating hyperglycemia may not be able to reverse this dysfunction. Additionally, every 1% increase in HbA1c level has been linked to an 8% increase in the risk of heart failure[5]. MicroRNA dysregulation is one of the pathogenic mechanisms of LVDD[6]. Approximately 22 evolutionarily conserved non-coding nucleotides comprise the family of naturally occurring, endogenous, single-stranded molecules that include microRNAs[7]. The primary regulators of gene expression in heart tissue that have recently been identified are cardiac miRNAs. Studies have demonstrated that these regulatory molecules impact transcriptional and post-transcriptional regulation in diabetic cardiomyopathy and heart failure[8]. Changes in the synthesis of miRNA and the quantities of specific miRNA are essential for cardiac remodeling and the development of heart failure[9].

The miRNAs may target several processes, such as mitochondrial function, ROS production, Ca2 + perturbation, apoptosis, fibrosis, pyroptosis, neuronal hormone secretion, and reactivation of a fetal gene program, which are thought to be important for the development of cardiac hypertrophy, remodelling, and heart failure[10]. Diabetes conditions have been found to cause a dawn regulation of miR-150 [11].

Left ventricular diastolic dysfunction can be treated with empagliflozin by its anti-inflammatory and anti- oxidant effect. Empagliflozin is a medication that inhibits SGLT2 and is approved for treating type 2 diabetes in adults in many regions, including the European Union, United States, and Japan. The EMPA-REG OUTCOME research, a significant cardiovascular outcomes trial (CVOT), showed that empaglifozin had cardioprotective and renoprotective effects that are mostly unrelated to glycaemic management in individuals with type 2 diabetes (T2D) and already present cardiovascular disease (CVD). The mechanism of action of empagliflozin is characterized by its powerful and highly specific inhibitor of SGLT2. It decreases the reabsorption of glucose filtered by the kidneys. It reduces the threshold at which glucose is reabsorbed, leading to increased urinary glucose excretion and decreased blood glucose levels. The glucuretic action is influenced by the levels of blood glucose and GFR (glomerular filtration rate), but it is not affected by insulin secretion or its activity.[12]

There is evidence indicating that the administration of Empagliflozin as an additional treatment with standard care resulted in a decrease in cardiovascular events, cardiovascular mortality, and the occurrence or progression of nephropathy in individuals with type 2 diabetes and pre-existing cardiovascular disease. Additionally, it was demonstrated that it decreased heart fibrosis in animal models of diabetes and hypertension. Empagliflozin effectively decreased the elevated production of TGF-β1 and the proteins Smad1, Smad2, and Smad3, which are implicated in the fibrosis pathway. Additionally, it lowered the excessive levels of type I and III collagen in the myocardium of diabetic mice. Furthermore, empagliflozin substantially enhanced the expression of Smad7, a well-known inhibitor of TGF-β1 and myocardial fibrosis[13]

MATERIAL AND METHOD

Study Design, Patient Recruitment, Setting, and Timing

This cross-sectional study was conducted from July 2023 to July 2024 at the Al-Diwaniyah Teaching Hospital in Diwaniyah, Iraq. This study included 60 patients with type 2 diabetes mellitus in total. All candidate's patients were diagnosed and recruited by a specialist caregiving physician.

Patient Inclusion Criteria

Type2 diabetes mellitus , HbA1C (6.5-9), Age 40-65 years, Onest of Diseases >3 years, Onest of current therapy > 6month

Patient Exclusion Criteria

HbA1C > 9, renal impairment, hepatic impairment, Pregnancy, Heart failure with reduced ejection fraction, Psychiatric patient,

Subjects

The study included 60 adults enrolled in this study (21 male and 39 female), aged 40-65 years diagnosed with type 2 diabetes mellitus.

Diagnosis of LVDD

Diagnosis and grading of LVDD was performed by transthoracic echocardiography utilizing ultrasound machine (Vinno E20, Echo Ultrasound AS, China) and the following cut off values indicating aberrant diastolic function were used to define LVDD per the most recent guidelines[14]. Patients who satisfied all of these criteria were diagnosed with abnormal diastolic function. Septal e' {septal mitral annulus velocity (early diastole) } is less than seven cm/s, and lateral e' {lateral mitral annulus velocity(early diastole)} is less than ten cm/s. Tricuspid regurgitation (TR) velocity is more than 2.8 m/ left atrial volume index greater than 34 milliliters per square meter.

Drug Used in The Study

Thirty patients included in the study took metformin1000 mg, and 30 others took empagliflozin 10 mg daily.

Ethical Approval

The University of Al-Qadisiyah's College of Medicine Ethics Committee approved the study. All patients were given a thorough explanation of the treatments, and their agreement was obtained before any work was done. All patients were informed verbally about the procedure and the study's purpose before enrolling.

Collection and Preparation of Samples

The blood samples with 1 ml were collected from the patients aspirated from the antecubital vein, placed in 1 ml EDTA tube, and refrigerated at a temperature of -20C until DNA extraction.

Genotyping

RNA isolation from a whole blood sample was then extracted using an RNA extraction kit from Genaid, Korea, to synthesize cDNA using a DNA synthesis kit from ADDBio, Korea.

Gene expression of Micro RNA150-5p

This approach was carried out according to the comparative Ct approach (ΔΔCt) with normalization to the level of the control group in the presence of the transcript levels to those of GAPDH mRNA. This was achieved according to the recommendation of (Schmittgen and Livak, 2008) [15].

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

Sample A means one certain group.

Sample B means another certain group.

The miR-150-5p gene was amplified using the primers listed below to achieve this.[16]

Table (1): Gene of Interest (miR-150-5p) [16]

Sequence (5'->3')	**Template strand (5'……3')**
miR-150-5p-Forward	TCAATGCCCTGTCTCCCAAC
miR-150-5p-Reverse	TTCCCAAGTCCCTATCCCCC

Table (2): Housekeeping gene (HKG) or internal reference gene; human Glyceraldehyde 3-phosphate dehydrogenase

Sequence (5'->3')	**Template strand (5'……3')**
GAPDH-F	CAGAACATCATCCCTGCCTCTA
GAPDH-R	CCAGTGAGCTTCCCGTTCA

RT-qPCR amplification

Initially, the amplification was achieved using AddScript RT-qPCR Syber master (AddBio, Korea) and
A/ the reaction was including:

Substance	Amount
H2O	Four µl
AddScript RT-qPCR	Ten µl
Forward primer (0.05 pmol/20 µl)	2 µl
Reverse primer (0.05 pmol/20 µl)	2 µl
cDNA	Two µl
Total	20 µl

Note: this was carried out for the internal reference gene (GAPDH) in the same components.

B/ The thermal conditions were carried out using BioRAD (USA) as follows:

Temperature		Time	Repeat
Initial denaturation	95 C	5 min	1
Denaturation	95 C	20 sec	40x
Annealing	55 C	30 sec	40x with machine-read
Extension	72 C	30 sec	40x
Melting analysis	95 C	15 sec	1
Melting analysis	60 C	60 sec	1
Melting analysis	+0.3 C of 95C	15 sec	With machine read

Statistical Analysis

Statistical analysis was done using SPSS (version 26) for echocardiographic parameters, while Gen expression of Micro RNA 150-5P was analyzed by Graph pad Prism software (version 8.4.3). P value was considered to be significant(p< 0.05). The data were expressed as mean ±SE

RESULT

Demographic Data

This study included 60 Iraqi type two diabetic patients; 66.6% (n = 40) were females, and 33.3% (n = 20) were males, with the mean ±SE Mean age (years) of the patient's Metformin group was 59.70± 1.986 and the mean ±SE Mean age (years) of the patient's Empagliflozin group was 49.39± 1.895

Echocardiographic Parameter

The mean ± SE mean of LVEDV(ml) ,LVESV(ml), EF(%),%FS of patients Metformin group were 124.1360 ± 6.36829 , 48.0007± 3.23524 , 61.9550± 0.88655 , 33.5960 ± 0.62250 respectively , and the mean ± SE mean of LVEDV(ml) ,LVESV(ML), EF(%),%FS of patients Empagliflozin group were 121.1333± 4.93090, 50.7258± 5.82147 , 62.7636±0 .67446, 34.1248± 0.50372 respectively

Table(3): values of Echocardiographic parameter of recruited diabetics patients (n = 60)

	LVEDV(ml)	LVESV(ml)	EF(%)	%FS
Metformin Mean	124.1360	48.0007	61.9550	33.5960
SE mean	6.36829	3.23524	0.88655	0.62250
Empagliflozin Mean	121.1333	50.7258	62.7636	34.1248
SE mean	4.93090	5.82147	0.67446	0.50372
P value	0.094	0.972	0.148	0.299

The assay amplification efficiency in tested groups for (miR-150-5p).

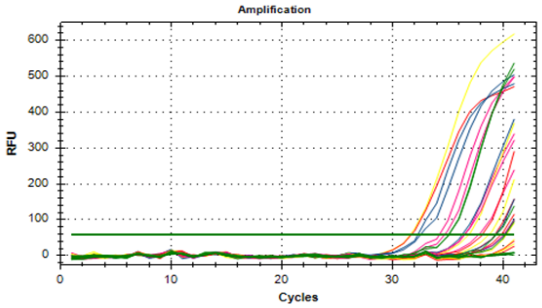

Figure (1): Amplification curve of the tested samples for expression of the gene of interest (miR-150-5p) in the Metformine group

The successful amplification curves with the corresponding crossing threshold (CT) are the number of cycles with the round forming unit (RFU).

Empagliflozin group

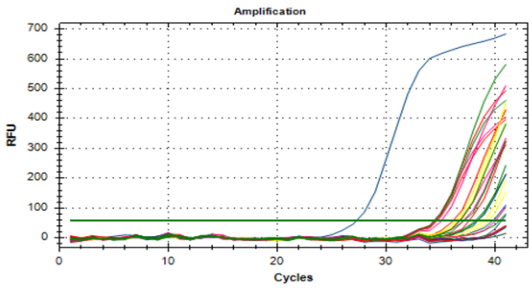

Figure (2): Amplification curve of the tested samples for expression of the gene of interest (miR-150-5p) in the Metformine group

The successful amplification curves with the corresponding crossing threshold (CT) are the number of cycles with the round forming unit (RFU).

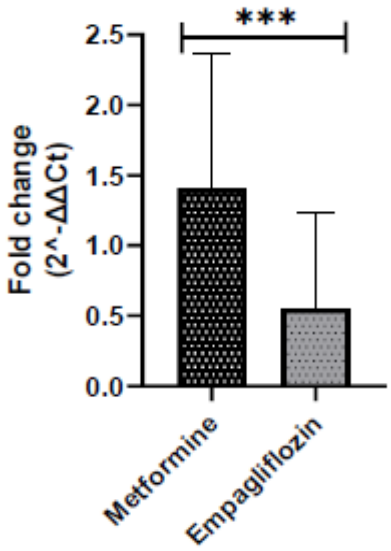

Figure (3): Gene expression of miR-150-5p in the metformin and empagliflozin treatment groups.

The figure shows a significant downregulation of miR-150-5p in the empagliflozin-treated group (P<0.001) compared to metformin treatment.

DISCUSSION

In this cross-sectional study, we examined the impact of Empagliflozin on micro RNA 150-5p in Iraqi diabetic patients with LVDD, and the findings revealed a significant decrease in miR-150-5p levels in the group treated with empagliflozin (P<0.001) compared to the control group receiving metformin. This suggests that empagliflozin negatively affects micro RNA 150-5p in diabetic patients.

According to other research, remodeling, hypertrophy, and apoptosis are some pathogenic mechanisms of heart failure in which microRNAs (miRNAs) are implicated[17]. Furthermore, differentially expressed miRNA patterns are linked to the etiology and the various stages of heart failure[18]. Extracellular circulating miRNAs exhibit remarkable stability, making them useful for heart failure diagnosis and prognosis and guiding therapeutic response. A study found that patients with heart failure had significantly lower normalized expression levels of miR-150-5p than patients without overt heart failure (p<0.001), suggesting a significant downregulation of miR-150-5p prior to acute decompensation[19].

Another study demonstrated a distinct pathway regulated by miRNA that controls the production p300 and the subsequent alterations observed in glucose-induced cardiomyocyte hypertrophy. By employing bioinformatics, researchers have predicted and confirmed that miR-150 is a potential p300 protein that specifically interacts with miRNA. Both in vivo and in vitro, elevated amounts of glucose resulted in the decrease of miR-150 levels and an elevation in p300 expression at the same time as an increase in the size of heart muscle cells (cardiomyocyte hypertrophy). Additionally, they have shown that the activation of PKCβ2 may be the primary mechanism responsible for the decrease in miR-150 levels caused by high hyperglycemia[11].

Empagliflozin plays an essential role in enhancing cardiac events because it has a positive effect on echocardiographic parameters, namely on LVEDV (ml), LVESV (ml), EF (%), and %FS. However, this effect is not statistically significant. However, its negative impact on micro RNA 150 -5p can be related to other factors such as place and temperature degree of storage, and this study has a number of limitations. For example, the sample size was small and single-center; most participants were women, and the patients adhered to the drug regimen.

CONCLUSION

1.Empagliflozin enhances cardiac events by improving several echocardiographic measures, such as left ventricular end-diastolic volume (LVEDV).

2.Empagliflozin causes a decrease in the expression of micro RNA in diabetic patients with left ventricular diastolic dysfunction, and this effect is statistically significant compared to metformin treatment.

Recommendation

More studies with larger sample sizes and family-based analysis are required to validate this effect. Future research should concentrate on the function of additional microRNA types in Iraqi diabetes patients.

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